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The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor : Umetaro SUZUKI.
Associate Editors : Kakuji GOTŌ and Yoshikazu SAHASHI.

Investigation on the Influence of Ultra-violet Rays on the Physiological Activities of Azotobacter. II.

On the Stimulation of Azotobacter Chroococcum by Ultra-violet Rays.

By

Arao ITANO and Akira MATUURA.

(Received June 19, 1934.)

The preceding paper (Jour. Agr. Chem. Soc., Japan. X : 477∼485, 1934.) dealt with the lethal action of ultra-violet rays on Azotobacter chroococcum, and in the course of investigation, it was found that a short exposure stimulated the physiological activity of Azotobacter, and in this investigation, the following points were investigated and the results are reported : 1. Rate of stimulation by different length of exposure, determined by the number of cells and also by the change of pH in the medium ; 2. Mechanism of stimulation, the influence of ultra-violet rays on the physical properties of medium and their subsequent influence on Azotobacter ; 3. Manner of exposure, continuous or intermittent.

The method used is the same as described previously, using Hanovia mercury lamp as the source of ultra-violet rays and exposed Azotobacter chroococcum for different intervals in Ashby's solution medium placed in Erlenmyer flask of hard glass, and obtained the following results : 1. The greatest number of cells was obtained by one minute exposure, and the longer exposure caused the decrease of cells. The change of pH in medium was greatest on one minute exposure and the reaction became acid ; 2. It was ascertained that the increase of cells was brought about chiefly by the action of ultra-violet rays such other factors as the heat rays, visible rays and the change of physical properties of medium have little influence ; 3. The influence of ultra-violet rays on the physical properties of medium such as electrical conductance, pH, osmotic pressure, viscosity and surface tension, was determined and found that the change of electrical conductance, pH and osmotic pressure was comparatively greater than that of other properties, and the reducing action was noted, although it is not sufficient to consider the reduction as an important factor which brings about the stimulation ; 4. So far as the manner of exposure is concerned, the better stimulation was obtained by the continuous exposure than by the intermittent within one minute, but when the exposure was longer, the intermittent exposure was better owing to the lethal action of continuous exposure.

Studies on the Digestion of the Carbohydrates Contained in Mulberry Leaves by Silkworms. Part II.

On the Digestibility and the Quantity of the Digestion of the Carbohydrates Contained in Mulberry Leaves in Different Stages of Growth.

By

Kiyotoki KATO.

(At the Sericultural Experimental Insititute of Kumamoto Prefecture.)

(Received June 23, 1934.)

E. Hiratsuka had been studied on the digestion of the carbohydrates contained in mulberry leaves by silkworms already. Writer also have been studied on this problem and reported in the first paper. On this problem there are many important matters to be studing on the standpoint of solving the problems of mulberry leaves nature. Now, writer would to be studing on these problems continuously. This report is the experimental results on the digestibility of the carbohydrates contained in mulberry leaves in different stages of growth.

Results are as follows.

(1) The digestibility and the digested quantity of the carbohydrates (total soluble carbohydrate, reducing sugar and canesugar in each) contained in mulberry leaves by silkworms vary with the stages of growth of mulberry leaves, namely, in younger and older leaves are small, middle leaves is larger. The digestibility of the carbohydrates is found to be correlated to the quantity of the corbohydrates contained in the leaves.

(2) The accumulated quantity of the fat and glycogen in silkworms and silkworm-chrysalis varies with the different stages of leaves, and this relation is similar to the relation of the digestibility of carbohydrates with the growth of leaves.

Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (IV).

Discussion of the Relation Among *β-Pacyman* and Related Compounds.

By

Kenzi TAKEDA.

(*Agricultural Chemical Laboratory, Tottori Agricultural College, Tottori.*)

(Received June 28, 1934.)

A study was made of the physical and the chemical properties of β-pachyman isolated from "Bukuryo", from the standpoint of the comparison with those of the closely related compounds, some of which have been considered by certain investigators, as identical, and the following conclusion has been made.

The author's β-pachyman is confirmed to be an independent carbohydrate, different from fongose (Tanret), callose (Mangin) or paradextran (Winterstein), and this should be likely, on the contrary, accepted as the same, in essence, as pectose (Gore), pachymose (Champion and Winterstein) and a carbohydrate which Wang and Takao isolated from "Bukuryo" in the different method from the case of the author's β-pachyman.

Moreover, it was found to be true that Tanret's fongose, at least, consists of two kinds of sugars, fongose I and II.

Chemical Studies on "Bukuryo", Sclerotia of *Pachyma Hoelen* RUMPH. (V).

Nutritive Value of *Pachyman*.

By

Kenzi TAKEDA.

(Received June 28, 1934.)

Albino rats were used and the digestion coefficient was studied with three kinds of "Bukuryo" preparations which were obtained by various methods of heating. The first was heated at 40° in water for 1.5 hours and

this treatment was repeated four times (A). In the second case, the temperature was the only factor which was varied, the water being brought to the boiling point (B). The third was autoclaved at 120° in double atmospheric pressure for 1.5 hours (C). These three samples were respectively filtered, washed throughly with water, dried and used for the feeding experiments. The composition of the rations used were as follows:

Ration I.

"Bukuryo" preparation	14.0
Casein (Merck)	14.0
Fat free dried beef powder	4.0
McCollum's salt	3.0
Cod liver oil	3.0
Oryzanin	2.0

Ration II.

Ration I (C)	50.0
Dried white bread	50.0

From two to five rats were used for each ration and the observation was made for each individual rat with the following conclusions.

(1) No remarkable difference in digestion coefficient could be found among these three kinds of pachyman, through the use of treated "Bukuryo", though the gradual increase of the digestion coefficient was recognized, as the temperature rose.

(2) When the content of pachyman in the ration (dry matter) was 60 ~66% and the nutritive ratio was narrow, ranging from 3.5 to 3.6 and no soluble sugar was contained, the maximum digestion coefficient of pachyman was in all cases lower than 55%.

(3) When the ration II which contained appreciable amount of digestible carbohydrate was used, the digestion coefficient of pachyman became extremely low, i. e. 18%.

(4) The nutritive value of pachyman resembled somewhat that of cellulose, and therefore, it can not be used in place of other soluble polysaccharides such as dextrin, starch or glycogen.

(5) Poor nutritive value of pachyman, which is the principal constituent of "Bukuryo" and the small amount of effective nutrients, make the nutritive value of "Bukuryo" itself less valuable, from the standpoint of nutrition, and it is highly probable that this can not be used as substitute for cereal grains.

On the Non-identity of Adenine and Vitamin B_4.

By

C. Y. Chen.

(*From the agricultural Chemical Laboratory, Tokyo Imperial University, Komaba, Tokyo.*)

(Received June 8, 1934.)

In a recent publication R. Tscheshe[1] has directed attention to the similarity between adenine hydrochloride and the crystals isolated by Barnes and Reader[2] which were stated by them to have vitamin B_4 potency (10 r per diem per rat). Later, Heard, Kinersley, Peter and Reader[3] showed that B_4 is not identical with adenine, Reader tested a specimen of adenine hydrochloride for B_4 activity with negative results. Neither did irradiated adenine produce a vitamin B_4 response.

The present author has also carried out some feeding experiments with albino-rats and come to the conclusion that adenine has no B_4 potency. The details are given in the following.

Experimental.

The adenine preparation used in this experiment was kindly furnished by Dr. S. Ohdake in this laboratory. It was isolated from dried brewers yeast and was proved to be quite pure. It melted at 360～365° and gave the characteristic purple red coloration by gently heating with zinc and hydrochloric acid on a water bath.

The experimental procedure was essentially the same with that outlined by Reader[4]. Young albino rats weighing 50～55 g were kept, each separately, in metal cages provided with wire-screen false bottoms. The Vitamin B_4 free basal diet was also prepared according to the direction of Reader[4] with certain modification. It was composed of purified casein, 20% ; purified starch, 75% ; McCollum's salt mixture, 3% ; and cod liver oil, 2%.

The casein was prepared from commercial product by dissolving in dilute ammonium hydroxide and precipitating with dilute acetic acid according to a modification of the method of Bosworth and Van Slyke[8]. The precipitated casein was then held for 5 days in 0.01% acetic acid, the acid being changed at least once a day. Next the casein was treated with 95% ethyl alcohol, and finally extracted with ether. The resultant white, friable, tasteless product was dried in a current of warm air and ground to a fine powder.

The starch was extracted for three days with 95% ethyl alcohol and then with ether to remove water soluble vitamins. It has been shown to be pure enough for this purpose. The cod liver oil was incorporated in the basal diet which was stored in a refrigerator for daily use.

Besides, the Vitamin B_2 used in the present investigation was prepared by a method described by T. Naraywan and Drummond(6) and 15 mg per day per rat were found to be sufficient. Further, oryzanin concentrate prepared by Dr. Ohdake was used as the source of B_1. This preparation was highly active and quite free from B_2 or B_4. One rat unit or approx. 0.002 mg being given to each rat per os, daily. The experimental animals were divided into two series, i. e. the curative and preventive. In the former, the rats were first fed on a basal diet alone until the typical symptoms of B_4 deficiency were developed, i. e. the decrease of body weight, with spastic gait, a tendency to sit in a hunched position etc. as described by Reader(4)(5). At that time adenine was administered at the rate of 20 mg per rat per day. In the latter, the animals were fed on the same basal diet supplementing adenine from the beginning of the experiment. For this purpose a definite amount of adenine was mixed with a small quantity of the basal diet and given to each rat. The rats were very soon found to consume this ration without objection.

Results.

The results of the experiments are shown in Figs. I, II, III and IV. From these observations, it must be concluded that adenine is ineffective in

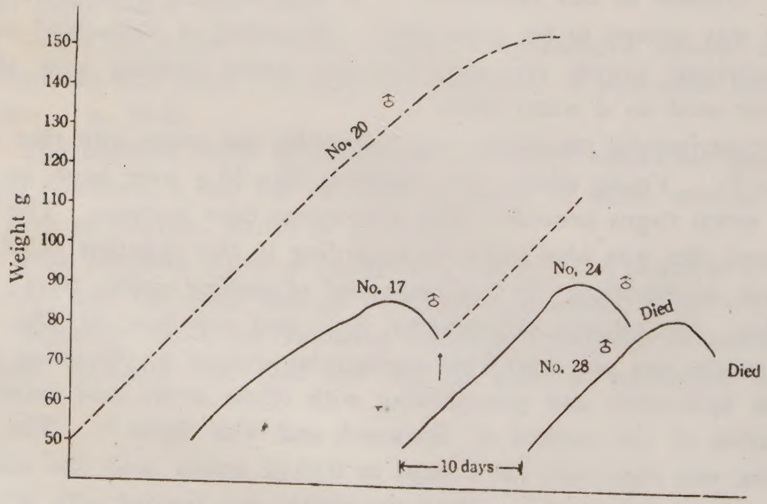

Fig. I. Shows the growth curves of rats fed on a diet in which Adenine was the sole source of Vitamin B_4.

———— Growth curves of the rat fed on a basal diet supplemented with Adenine.
-------- Adenine was replaced with 0.4 g of dry yeast daily.
—-—-—- Basal diet supplemented with 5% dry yeast.

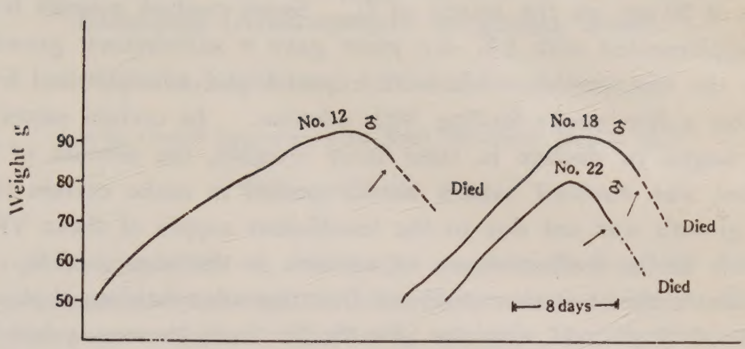

Fig. II. Shows the growth curves of rats fed on a B_4-free diet, Adenine or Adenyl-thio-methyl-pentose were supplied as the source of B_4, at the point marked by the arrow in each group.

———————— Growth curves on a basal diet alone.
---------------- ditto, supplemented with 20 mg. Adenine daily.
-·-·-·-·-·-·-·- ditto, supplemented with 0.05 g. Adenyl-thio-methyl-pentose.

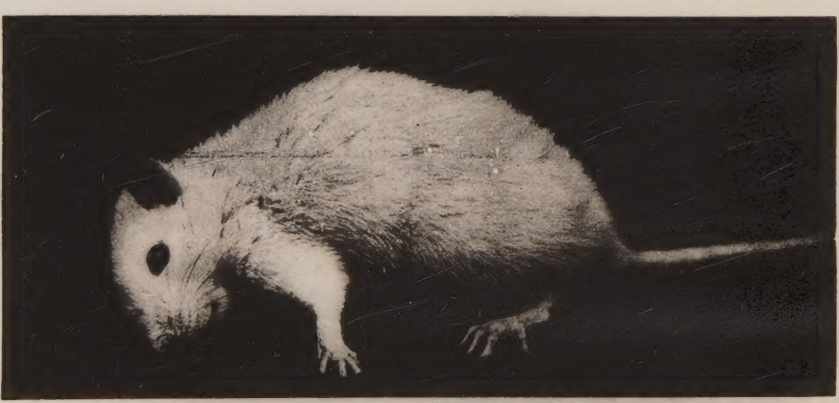

Fig. III. Rat 17 after 10 weeks on a basal diet supplemented with 20 mg of Adenine daily. Weight, 77 g.

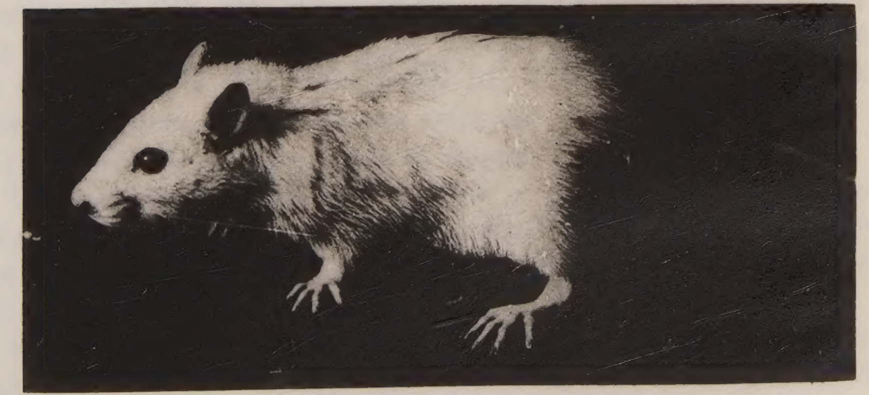

Fig. IV. The same Rat 10 days after receiving 0.4 g dry yeast daily. Weight, 113 g.

a daily dose of 20 mg, as the source of B_4. Some control animals fed on a basal diet supplemented with 5% dry yeast gave a satisfactory growth response within the test period, while both experimental animals died from B_4-deficiency after a few weeks feeding with adenine. In certain cases, when the animals began to decline in their body weights, the amount of B_1 and B_2 in the diet was doubled over a weekly period to make certain that the cessation of growth was not due to the insufficient supply of these Vitamins, but simply due to the ineffectiveness of adenine as the source of B_4.

The present author has also observed that the adenyl-thiomethyl-pentose, isolated by Prof. U. Suzuki, Ohdake and Mori(7) from brewers yeast has no B_4-activity.

Summary.

Pure Adenine, prepared from dry yeast does not serve as a source of Vitamin B_4 in the albino-rat. Since it has been shown repeatedly that the feeding of large amounts of adenine neither prevents nor cures the typical symptoms of Vitamin B_4 deficiency.

My thanks are due to Proffesor U. Suzuki for his advice and interest throughout the work to Dr. S. Ohdake for the supply of oryzanin, Vitamin B_1 concentrates and to Mr. H. Ariyama for his continued help in this experiment.

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On the Isolation of Apigenin from Manchurian Kaoliang (Andropogon sorghum, Brot).

Koji OKANO, Tadao ABE and Iwao OHARA.

(*At the Central Laboratory of the South Manchuria Railway Co.*)

(Received July 25, 1934)

The colouring matters of *Andropogon sorghum*, from which durasantalin ($C_{16}H_{12}O_5$) as a natural substantive dyestuff was obtained by A. G. perkin(1 (he employed var. vulgaris "red dura"), are now investigated and yellow needle crystals, melting at 347°C, are isolated. From the analytical numbers of the acetyl- and methyl-derivatives, the decomposition products and the absorption spectrum in ultra-violet rays of the crystal it is established that the substance is apigenin or 5, 7, 4′-trioxyflavone itself.

Experimental.

(1) Isolation of apigenin.

The material, the hulls of Manchurian Kaoliang [*Andropogon sorghum, Brot* (*Kaoliang*)] is digested with boiling methyl alcohol three times to extract the colouring matters yielding 7～8%. The deep purple-red concentrated extracts are poured into ether. After evaporation to dryness of ether apigenin is obtained from insoluble part in acetone as a fine purple-red mass. The yield is about 12% of all colouring matters, corresponding to about 1% of the raw materials. The acetone-soluble part or other colouring matters will be communicated in next paper.

For purification of apigenin, lead acetate is added in its alcohol solution to remove the impurities as precipitates. On evaporation of filtrates after treatment with SH_2, the minute straw-coloured crystals are separated. A small amount of calcium is so tightly adhesived to the above obtained crystals that is treated with concentrated HCl to remove and is sublimated in vacuum, then pure crystals are now collected.

(2) Analysis and derivatives of apigenin.

The apigenin thus obtained consists of pale yellow coloured needles, melting at 347°C, soluble in alcohol, methyl alcohol and pyridin. Its alcohol solution gives a purple-black colour with ferric chloride and dilute alkalis dissolve it with a yellow colouration.

Its derivatives, triacetyl, diacetyl, dimethyl and monomethyl, are now obtained by ordinary methods and those analytical numbers are shown as follows :

		apigenin	triacetyl 〃	diacetyl 〃	dimethyl 〃	monomethyl 〃
mel. pt. (°C)		347	185	198	172	168
C %	cal.	66.67	63.64	64.41	68.46	67.41
	obs.	66.65	62.59	64.30	68.32	67.42
H %	cal.	3.70	4.04	3.95	4.70	4.23
	obs.	3.94	4.04	3.85	4.71	4.32
methoxyl %	cal.				20.80	10.92
	obs.				20.36	10.28
mol. wt.	cal.		396	354	298	284
	obs.		364	341	313	262

The melting point of the triacetyl derivatives shows higher than that in the reports by Czajkowski(2) or L. Schmidt(3), which melts at 181~182°C, but others are identical.

(3). Decomposition of apigenin with alkalis.

(A) By digestion with the gentle action of alkalis (20% KOH for 2 hours), *p*-oxyacetophenone and phloroglucin are separated.

(B) Action of fused alkalis at 220~260°C results the formation of phloroglucin, *p*-oxybenzoic acid and acetic acid.

(4) The absorption spectrum of apigenin.

The absorption spectrum of apigenin in ultra-violet rays is now photographep and we observed its characteristic absorption, but in this paper it is spared.

Literature.

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Investigation on Agar. (Continued). Physico-chemical Properties of Agar and their Influence on the Growth of Microorganisms.

By

Arao ITANO and Y. TUJI.

(Received July 10, 1934)

The previous reports(1, 2 & 3) dealt with the general review on agar as to the seaweeds from which the agar is manufactured and the iodine contents and also the history together with general chemical composition. In this investigation, the physico-chemical properties of agar of different grades I, II and III on the market, were determined and their subsequent influence on the growth of Azotobacter chroococcum, Bacillus subtilis and Saccharomyces cerevisiae was tried.

The following results were obtained: (1) Better the quality of agar is, contained less nitrogen and ash, and also faster the coagulation and solubility; (2) So far as the specific gravity, specific conductance, osmotic pressure and surface tension of the liquid agar and the filtered agar are concerned, better the quality of agar is, the value of these properties was smaller while the concentration of hydrogen ions of the first grade was greater than the others, and the unfiltered, liquefied agar was more acidic than the filtered agar.

The influence of agar of different grade, I and III was tried on Azotobacter chroococcum, B. subtilis and Saccharomyces cerevisiae, by cultivating them on the solid medium of known quality with an addition of I or III grade agar for each organism, namely Ashby's, Laurent's and the nutrient agar medium respectively. In all these cases, the organism grew better on the third grade agar and especially it was marked in case of Saccharomyces cerevisiae.

From the results thus obtained, it may be concluded that the agar of different grade differ markedly in regard to the physicochemical properties, and when they are used for cultivation of microorganisms as the solidifying agent, they influence the growth of microorganisms differently, and the agar of lower grade supports the better growth which may be due to the presence of a larger quantity of some stimulating substance such as iodine and others. Consequently it is very important to use the purified agar in order to obtain the uniform and comparative results in the microbiological investigation.

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Untersuchungen über die Enzyme von Bombyx mori L. VII. Mitteilung.

Über die Eierkatalase. (1).

Von

Kazuo YAMAFUJI.

(Aus dem Biochem. Institut der Landw. Abteilung der Kaiserl. Kyushu-Universität zu Fukuoka, Japan.)

(Eingegangen am 31, Juli 1934)

Trotzdem zahlreiche Angaben über die in der Tierwelt vorhandene Katalase verliegen, haben wir dennoch kein eingehenden Arbeiten dieses Enzyms bei Insekten. Da ich einige Eigenschaften der Katalase aus den Eiern von Bombyx mori aufgeklärt habe, schreibe ich hier einen deutschen Auszug aus dem japanischen Original, das im Journal of Agric. Chem. Soc. Jap. dargestellt wurde.

I. Gewinnung und Reinigung des Enzyms.

Ein Beispiel wird kurz angegeben: 850 g Eier wurden zerrieben, mit 500 ccm Wasser vermischt und 5 Stunden bei Zimmertemperatur belassen. Nach Absaugen wurden 750 ccm des Filtrates mit 370 ccm Alkohol versetzt und zentrifugiert. Die Restlösung wurde mit 270 ccm Chloroform geschüttelt und die Fällung durch Zentrifugieren abgetrennt. Zur wässerigen Lösung wurden 300 ccm 1,5 proz. $Ca_3(PO_4)_2$-Suspension zugesetzt und nach kurzem Stehen wurde das Adsorbat gesammelt. Dann wurde die Katalase mit 100 ccm M/15-Na_2HPO_4-Lösung eluiert. Die von dem Adsorbens abzentrifugierte Katalaselösung wurde im Kollodiumsack 20 Stunden lang bei 0° dialysiert und unter Chloroformzusatz im Eisschrank aufbewahrt. Kat. f. der Eier wurde auf 7 bestimmt und die der gereinigten Enzymlösung auf 280.

II. Bestimmung der Enzymwirkung.

25 ccm 0,01 n-H_2O_2-Lösung, 1 ccm M/5-Phosphatpufferlösung von pH 6,8, Enzymlösung und Wasser wurden vermischt, und zwar betrug das Totalvolumen stets 30 ccm. Die Versuchstemperatur war 0°. Nach 0, 5, 10 und 15 Minuten wurden 5 ccm von der Reaktionsmischung abpipettiert und in 10 ccm 1 n-H_2SO_4 eingelassen. Dann wurde das nicht gespaltene H_2O_2 mit 0,005 n-$KMnO_4$-Lösung titriert. Die Aktivität der Katalase wurde mit den nach der Formel für monomolekulare Reaktion berechneten Reaktionskonstanten verglichen.

III. Reaktionskonstanten und Enzymmengen.

Enzymmenge, E	1		4		8	
Zeit, Min.	$KMnO_4$, (ccm)	$k.10^4$	$KMnO_4$, (ccm)	$k.10^4$	$KMnO_4$, (ccm)	$k.10^4$
0	9,00	—	9,00	—	8,98	—
5	8,70	29	7,92	111	7,10	203
10	8,49	25	7,18	98	5,82	188
15	8,24	24	6,53	93	5,03	168
Mittel	—	26	—	101	—	186
$k.10^4/E$	—	26	—	25	—	23

IV. Einfluss der Wasserstoffionenkonzentration auf die Enzymwirkung.

pH	5,3	6,2	7,0	8,0
$k.10^4$	78	137	149	146
pH	6,6	6,8	7,2	7,7
$k.10^4$	149	167	157	151

V. Einfluss der Temperatur auf die Enzymwirkung.

1) *Optimale Temperatur.*

Temp.	20	25	30	35
$k.10^4$	175	186	177	170

2) *Temperaturkoeffizient.*

Temp.	$k.10^4$	Temp.-Gebiet	$kt_{+10}:kt$	A
0	127	——	——	——
10	148	0~10	1,17	2426
20	166	10~20	1,12	1878
30	178	20~30	1,07	1202

Die Konstante A wurde nach der Arrheniusschen Formel $\ln(kt_1/kt_2)=A(T_1-T_2)/R \cdot T_1 \cdot T_2$ berechtet.

VI. Thermostabilität des Enzyms.

1) *Tötungstemperatur.*

Erhitzungsdauer 60 Min., pH 6,8.

Erhitzungstemp.	Nicht erhitzt	50	55	60	70	80
$k.10^4$	145	94	75	39	15	7
Rel. k	100	65	52	27	10	5

2) *Abhängigkeit der Inaktivierung von der Enzymkonzentration.*

Erhitzungsdauer 60 Min., pH 6,8.

Verdünnnng	Nicht erhitzt	Erhitzte, Erhitzungstemp. 55°		
	1 : 1	1 : 1	1 : 5	1 : 9
$k.10^4$	136	78	75	54
Rel. k	100	57	55	40
$kc.10^4$	——	40	43	67

Die Inaktivierungskonstante kc wurde nach der Gleichung $kc = 1/t \log(ka/kt)$ berechnet.

3) *Abhängigkeit der Inaktivierung von der Azidität.*

Erhitzungstemperatur 50°. Zeit 60 Min.

pH	$k.10^4$	pH	$k.10^4$
5,3	79	6,6	85
6,6	86	6,8	89
8,0	54	7,0	81

4) *Zeitlicher Verlauf der Inaktivierung.*

Erhitzungstemperatur 55°, pH 6,8.

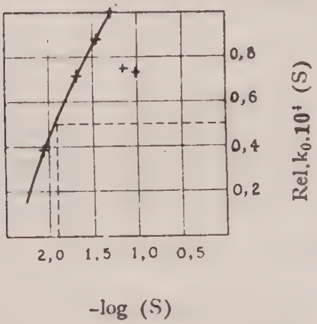
Erhits.-Dauer, Min	0	30	60	90
$k.10^4$	113	75	59	49
Rel. k	100	66	52	43
$kc.10^4$	——	59	47	40

VII. Affinitätskonstante der Verbindung Enzym-substrat.

〔S〕, m	-log 〔S〕	$k_0.10^4$	$k_0.10^4$. 〔S〕	Rel.$k_0.10^4$. 〔S〕
0,0086	2,066	198	1,70	0,38
0,0206	1,686	152	3,13	0,71
0,0344	1,463	111	3,82	0,87
0,0495	1,305	89	4,41	1,00
0,0698	1,156	47	3,28	0,74
0,0942	1,026	33	3,21	0,73

〔S〕 bedeutet die Konzentration des Substrats und k_0 die Reaktionskonstante in der Zeit 0.

Aktivitäts-pS-Kurve



log Km = −1,925

Km = 0 0119

1/Km ≑ 84

Zusammenfassung.

(1) Unter Anwendung der Adsorptionsmethode mit Hilfe des Calciumphosphats wurde aus den Eiern von Bombyx mori ein Katalasepräparat hergestellt.

(2) Die monomolekularen Reaktionskonstanten der Katalasewirkung in 0,01 n-H_2O_2-Lösung bei 0° fallen allmählich. Aber zwischen den Enzymmengen und Reaktionskonstanten besteht eine gute Proportionalität.

(3) Die Eierkatalase wirkt am besten bei pH 6,8; ihre Optimaltemperatur liegt bei 25°.

(4) Der Temperaturkoeffizient $kt_{+10} : kt$ und die Konstante A der Arrheniusschen Temperaturformel zwischen 0 bis 10° sind 1,17 und 2400. Bei höherer Temperatur nehmen diese Werte ab.

(5) Die Tötungstemperatur der Katalase liegt bei etwa 55°. Nach 60 Minuten Erhitzung bei 80° ist die Aktivität nahezu auf Null gesunken.

(6) Die Katalase zeigt die maximale Thermostabilität bei pH 6,8 und diese Stabilität nimmt bei steigender Enzymkonzentration zu.

(7) Die Inaktivierung der Eierkatalase bei Erhitzung verläuft nicht monomolekular, sondern die Konstante kc der monomolekularen Reaktion nimmt ab.

(8) Als Affinitätskonstante für die Verbindung Enzym-Substrat wurde 84 bestimmt.

Untersuchungen über die Enzyme von Bombyx mori L. VIII. Mitteilung.

Über die Eierkatalase (2).

Von

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(Eingegangen am 31. Juli 1934)

Bezüglich der Einflüsse der äusseren Faktoren aus die Katalasewirkung ist bereits von zahlreichen Forschern mitgeteilt worden. Um eingehendere Kenntnisse über die Hemmungsrescheinungen der Katalase durch chemische Stoffe zu gewinnen, habe ich nun die Salzwirkung auf das Enzym studiert.

I. Gewinnung und Reinigung des Enzyms.

800 g der fein gemahlenen Eier wurden mit 400 ccm Wasser 3 Stunden lang bei Zimmertemperatur extrahiert. Der Extrakt wurde über Nacht unter Chloroformzusatz im Eisschrank aufbewahrt und dann mit 250 ccm Alkohol und 200 ccm Chloroform unter Kühlung kräftig geschüttelt. Nach Abzentrifugieren der Fällung wurde die wässerige Lösung mit 300 ccm 3 proz. $Al(OH)_3$-Suspension, die nach der Vorschrift von Willstätter und Racke (Liebigs Ann. 425, 1.) bereitet wurde, versetzt. Die 150 ccm betragende Elution, welche mit 1 proz. sekundärem Natriumphosphat erhalten worden war, wurde während 1 Tages im Eischarnk dialysiert. Die Eier zeigten Kat. f. etwa 7 und die gereinigte Katalaselösung 270.

II. Einrichtung der Hemmungsversuche.

Die Versuche wurden mit Reaktionsmischungen von 30 ccm Totalvolumen ausgeführt. 2,5 ccm 0,1 n-H_2O_2, 1 ccm M/5-Phosphatpuffer von pH 6,8, 1 ccm Enzymlösung und Salzlösung wurden vermischt und mit Wasser auf 30 ccm aufgefüllt. Die Versuchstemperatur wurde stets auf 0° gehalten. Zur Bestimmung der Katalaseaktivität wurde wie früher die Permanganatmethode angewandt, und auch als Mass der Hemmung die Reaktionskonstante erster Ordnung gewählt.

III. Hemmung durch Natriumsalze.

	Salzkonz. n	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
NaCl	$k.10^4$	160	—	164	156	152	119	112
	Rel.k	100	—	102	97	95	74	70
NaF	$k.10^4$	158	—	—	—	135	70	15
	Rel.k	100	—	—	—	85	44	9
$NaNO_3$	$k.10^4$	158	142	—	111	—	94	48
	Rel.k	100	90	—	71	—	60	31
Na_2SO_4	$k.10^4$	155	155	—	154	—	150	—
	Rel.k	100	100	—	100	—	95	—
Na_3PO_4	$k.10^4$	151	—	153	—	148	—	146
	Rel.k	100	—	101	—	98	—	96
Na_2S	$k.10^4$	167	130	76	10	—	—	—
	Rel.k	100	77	45	6	—	—	—
NaSH	$k.10^4$	173	—	90	19	14	—	—
	Rel.k	100	—	52	11	8	—	—
NaCN	$k.10^4$	145	132	19	3	—	—	—
	Rel.k	100	84	13	2	—	—	—
Na-Azetat	$k.10^4$	162	130	—	127	—	121	62
	Rel.k	100	80	—	78	—	75	44
Na-Butyrat	$k.10^4$	140	—	—	145	146	134	—
	Rei.k	100	—	—	104	104	96	—
Na-Lactat	$k.10^4$	154	141	—	133	—	124	80
	Rel.k	100	92	—	86	—	80	52
Na-Oleat	$k.10^4$	151	131	96	42	18	—	—
	Rel.k	100	87	64	28	12	—	—

Aus den oben erwähnten und anderen Data wurden durch graphische Intrapolation diejenigen Konzentrationen der Salze ermittelt, die die Katalasewirkung auf die Hälfte herabsetzen ($H_{50\%}$).

Salz	$H_{50\%}$, n	Salz	$H_{50\%}$, n	Salz	$H_{50\%}$, n
Butyrat	$> 10^{-2}$	Lactat	10^{-1}	Oleat	5.10^{-5}
Sulfat	$>8.10^{-1}$	Azetat	8.10^{-2}	Hydrosulfid	10^{-5}
Phosphat	7.10^{-1}	Nitrat	2.10^{-2}	Sulfid	9.10^{-6}
Chlorid	6.10^{-1}	Fluorid	8.10^{-3}	Cyanid	3.10^{-6}

IV. Hemmung durch Kaliumsalze.

	Salzkonz. n	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
KCl	$k.10^4$	149	—	—	133	—	105	100
	Rel.k	100	—	—	89	—	70	67
KNO_3	$k.10^4$	149	149	—	122	—	84	39
	Rel.k	100	100	—	82	—	56	27
K_2SO_4	$k.10^4$	135	136	—	136	—	—	125
	Rel.k	100	100	—	100	—	—	93
K_3PO_4	$k.10^4$	152	—	153	—	148	—	136
	Rel.k	100	—	100	—	97	—	89
K_2S	$k.10^4$	136	123	85	25	—	—	—
	Rel.k	100	90	63	19	—	—	—
KCN	$k.10^4$	130	118	12	3	—	—	—
	Rel.k	100	91	9	2	—	—	—
K-Azetat	$k.10^4$	147	—	148	—	148	112	49
	Rel.k	100	—	100	—	100	76	33
K-Butyrat	$k.10^4$	145	—	—	139	136	134	—
	Rel.k	100	—	—	96	94	92	—

Salz	$H_{50\%}$, n	Salz	$H_{50\%}$, n
Butyrat	$>3.10^{-2}$	Azetat	6.10^{-2}
Sulfat	$>6.10^{-1}$	Nitrat	3.10^{-2}
Phosphat	7.10^{-1}	Sulfid	4.10^{-5}
Chlorid	4.10^{-1}	Cyanid	5.10^{-6}

Zusammenfassung.

Es wurden die hemmende Wirkungen den verschiedenen Natrium- oder Kaliumsalze auf die durch Tonerdeadsorption gereinigte Eierkatalase geprüft. Dabei ergab sich, dass der Effekt der Salze hauptsächlich auf ihre Anionen zurückzuführen ist und zwar die folgende Reihenfolge von schwacher zu starker Hemmung besteht: Butyrat $< SO_4 < PO_4 <$ Cl $<$ Lactat $<$ Azetat $< NO_3 <$ F $<$ Oleat $<$ SH $<$ S $<$ CN.

Untersuchungen über die Enzyme von Bombyx mori L. IX. Mitteilung.

Über die Proteasen und Amylase des Blutes (2).

Von

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(Eingegangen am 31. Juli 1934.)

Obwohl die eiweissspaltende Reaktion des Blutes der Seidenraupe zwei ausgeprägte pH-Optima zeigt, so ist noch nicht einwandfrei festgestellt worden, dass im Blute derselben zwei Sorten Proteasen, nämlich Pepsinase und Tryptase vorkommen (Vgl. dieses Bull. 9, 19.). Deshalb wurden in der vorliegenden Arbeit vorläufig die Namen Protease-a und Protease-b gebraucht, wobei das Wirkungsoptimum der ersteren bei pH 2,3 liegt und das der letzteren bei pH 8,8.

I. Proteasen.

A. Protease–a.

Die Versuchsanordnung wurde diesmal gegenüber der früher von mir beschriebenen nur unwesentlich verändert. Die Substratlösung wurde dadurch bereitet, dass 5 g Kasein nach Hammersten mit 100 ccm 0,1 n-NaOH gelöst und mit Wasser auf 1000 ccm gefüllt wurden. Die Reaktion der Proben wurde mit 0,1 m-Sörensenschem Zitrat-HCl-Puffer während der Versuchszeit konstant gehalten. Als Enymlösung benutzte ich das mit 0,85 proz. NaCl-Lösung fünffach verdünnte Blut. 4 ccm 0,5 proz. Kaseinlösung, 5 ccm Pufferlösung von pH 2,3 und 1 ccm Enzymlösung wurden gemischt und während 24 Stunden bei 30° stehen gelassen. Die Ermittlung des Grades der Kaseinspaltung durch das Blut führte ich nach der einfachen nephelometrischen Methode durch. Zu diesem Zweck wurde in den meisten Fällen 0,2 ccm von der Reaktionsmischung entnommen und in 5 ccm 5 proz. Sulfosalicylsäure eingelassen. Die entstandene Trübung wurde mit der Standardtrübung, die durch Hinzusetzen der nicht gespaltenen Kaseinlösung zur 5 proz. Sulfosalicylsäure hergestellt wurde, mittels des Nephelometers verglichen. Zugleich wurden Blindproben ausgeführt.

1) Änderungen durch Hungerzustand bei den Larven.

Hungerstunden	♀		♂	
	Gespaltenes Kasein mg	Spaltung %	Gespaltenes Kasein mg	Spaltung %
0	8,98	44,9	10,00	50,0
24	7,72	38,6	8,04	40,2
48	6,28	31,4	6,00	30,0

2) Unterschied zwischen gut gewachsenen und schlecht gewachsenen Larven.

	Gut gewachs. Larven		Schlecht gewachs. Larven	
	♀	♂	♀	♂
Gespaltenes Kasein, mg	8,89	9,55	9,23	9,63
Spaltung, %	44,5	47,8	46,2	48,2

3) Veränderungen im Laufe der drei Entwicklungsperioden von Bombyx mori.

	Tage	♀		♂	
		Gespalt. Kasein mg	Spaltung %	Gespalt. Kasein mg	Spaltung %
Larve	V. Lebensalter 3	5,71	28,6	5,57	27,9
	6	7,61	38,1	8,62	43,1
	8	9,63	48,2	10,34	51,7
Einspinnen des Kokons	2	10,34	51,7	10,48	52,4
	4	8,24	41,2	8,43	42,4
Puppe	2	6,92	34,6	8,89	44,5
	6	8,71	43,6	9,71	48,6
	9	12,51	62,6	13,68	68,4
	11	11,36	56,8	13,33	66,7
Schmetterling	2	8,43	42,2	——	——

4) Unterschied zwischen den verschiedenen Rasen der Seidenraupen.

V. Lebensalter, Tage	3		5		8	
Rasse	Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %
Japan-111-E	6,48	32,4	10,24	51,6	10,28	51,4
Japan-110-G	5,64	28,3	8,12	40,6	9,99	50,0

China-7-B	9,01	45,1	10,87	54,4	11,83	59,2
China-7-D	9,13	45,7	9,19	46,0	11,23	56,2
Europa-7-A	7,04	35,2	9,79	49,0	9,35	46,8
Europa-7-C	9,24	46,2	10,76	53,8	11,10	55,6

B. Protease-b.

Die Versuche erfolgten mittels folgenden Verfahrens. Zur Mischung der 4 ccm 0,5 proz. Kaseinlösung und der 5 ccm M/15-Glykokoll-NaCl-NaOH-Pufferlösung von pH 8,9 wurde 1 ccm mit 0,85 proz. NaCl-Lösung fünffach verdünntes Blut hinzugefügt. Nach 24 Stunden Erwärmung bei 30° wurde wie früher die Abspaltung des Kaseins nephelometrisch ermittelt.

1) *Änderungen durch Hungerzustand bei den Larven.*

Hungerstunden	♀		♂	
	Gespaltenes Kasein mg	Spaltung %	Gespaltenes Kasein mg	Spaltung %
0	1,82	9,1	2,72	13,6
24	0,82	4,1	1,58	7,9
48	1,58	7,9	2,50	12,5

2) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Larven.*

	Gut gewachs. Larve		Schlecht gewachs. Larve	
	♀	♂	♀	♂
Gespaltenes Kasein, mg	2,28	1,58	2,72	2,93
Spaltung, %	11,4	7,9	13,6	14,7

3) *Veränderungen im Laufe der drei Entwicklungsperioden von Bombyx mori.*

	Tage		♀		♂	
			Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %
Larve	V. Lebensalter	3	8,33	41,7	8,43	42,2
		6	5,57	27,9	6,00	30,0
		8	3,72	18,6	5,71	28,6
Einspinnen des Kokons	2		2,28	11,4	3,53	17,7
	4		4,95	24,8	4,44	22,2

Puppe	2	4,95	24,8	4,95	24,8
	6	5,42	27,1	5,57	27,9
	9	7,61	38,1	7,61	38,1
	11	8,71	43,6	8,89	44,5
Schmetterling	2	2,50	12,5	——	——

4) *Unterschied zwichen den verschiedenen Rassen der Seidenraupens.*

V. Lebensalter, Tage	3		5		8	
Rasse	Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %	Gespalt. Kasein mg	Spalt. %
Japan-110-E	5,55	27,8	5,72	28,6	5,26	26,3
Japan-110-G	4,18	21,0	4,08	20,5	5,71	28,6
China-7-B	8,38	42,0	5,79	29,0	4,72	23,6
China-7-D	7,16	35,8	5,69	28,5	4,98	24,9
Europa-7-A	5,71	28,6	4,36	21,8	3,82	19,6
Europa-7-C	7,04	35,2	4,44	22,2	4,44	22,2

II. Amylase.

Zur Bestimmung der saccharifizierenden Wirkung des Blutes wurde immer 1 ccm mit 0,85 proz. NaCl-Lösung fünffach verdünntes Blut mit 5 ccm 5 proz. frisch hergestellter Stärkelösung und mit 1 ccm 0,2 m-Phosphatpuffer von 6,8 versetzt, mit Toluol überschichtet und für 24 Stunden in einen Thermostaten bei 30° gestellt. Nach Verlauf dieser Zeit wurde die Zuckerbestimmung nach Bertrand ausgeführt. Die Zahlen, durch die wie das Verzuckerungsvermögen des Blutes ausdrücken, bedeuten die Milligramme Zucker, die als Maltose berechnet werden. Der Versuch wurde auf jeden Fall am Tage der Blutentnahme angestellt.

1) *Änderungen durch Hungerzustand bei den Larven.*

Hungerstunden	♀		♂	
	Zucker, mg	Spaltung, %	Zucker, mg	Spaltung, %
0	32,2	12,2	31,5	12,0
24	46,5	17,7	36,8	14,0
48	45,0	17,1	44,1	16,8

2) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Larven.*

	Get gewachs. Larve		Schlecht gewachs. Larve	
	♀	♂	♀	♂
Zucker, mg	37,3	31,9	36,8	31,5
Spaltung, %	14,2	12,1	14,0	12,0

3) *Veränderungen im Laufe der drei Entwicklungsperioden von Bombyx mori.*

	Tage		♀		♂	
			Zucker, mg	Spalt. %	Zucker, mg	Spalt. %
Larve	V. Lebensalter	3	25,3	9,6	25,3	9,6
		5	33,9	12,9	31,5	12,0
		8	49,5	18,8	37,0	14,1
Einspinnen des Kokons	3		57,3	21,8	50,8	19,3
	5		54,7	20,8	53,9	20,5
Puppe	4		44,3	16,8	42,6	16,2
	8		41,3	15,7	35,0	13,3
	11		39,9	15,2	31,1	11,8
	13		30,8	11,7	25,6	9,3
Schmetterling	2		42,3	16,1	——	——

4) *Unterschied zwischen den verschiedenen Rassen der Seidenraupen.*

V. Lebensalter, Tage	3		5		8	
Rasse	Zucker mg	Spalt. %	Zucker mg	Spalt. %	Zucker mg	Spalt. %
Japan-110-E	13,5	5,1	17,5	6,6	20,8	8,0
Japan-110-G	20,3	7,6	25,5	9,7	27,3	10,4
China-7-B	25,3	9,6	32,7	12,5	43,3	16,5
China-7-D	17,6	6,2	24,9	9,5	28,2	10,7
Europa-7-A	25,5	9,7	32,3	12,3	37,4	14,2
Europa-7-C	27,0	10,3	33,1	12,6	47,3	18,0

III. Diskussion.

Durch eine Reihe früher mitgeteilter Versuche, die Änderungen des Fermentgehaltes des Blutes bei den verschiedenen physiologischen sowie pathologischen Bedingungen von Bombyx mori zum Gegenstand haben, dürfte die Abhängigkeit einiger Blutfermente vom Stoffwechsel in eindeutiger Weise festgestellt sein. Es konnte nämlich vor allem in den fünften und sechsten Mitteilungen gezeigt werden, dass die Saccharase, Maltase und Lipase im Blute dieses Insekts bei den Umsetzungen der Disaccharosen und des Fettes in seinem Körper eine wichtige Rolle spielen.

Diese Kenntnis enger Beziehungen dieser drei Bluthydrolasen zu den Umwandlungsvorgängen der Stoffe im Körper hat mich weiterhin zu der Vermutung geführt, dass auch die Amylase und Proteasen des Blutes bei Bombyx mori am Kohlenhydrat- bzw. Eiweissstoffwechsel· Anteil nehmen müssen. Zu Gunsten dieser Vorstellung scheinen mir die in der vorliegenden Arbeit angestellten Experimente zu sprechen.

Ich beobachtete, dass die Rassen, in deren Digestionssaft keinen oder nur wenige Amylase vorkommt, recht viel stärkeres amylolytisches Ferment im Blute enthalten, als diejenigen Rassen, die eine viel grössere stärkespaltend Kraft der Verdaungsflüssigkeit besitzen. Es erscheint biologisch äusserst unwahrscheinlich, dass selbst bei der Seidenraupe ihre Magenwand hochmolekulare Nährstoffe, wie Stärke oder Eiweiss, durchlassen sollte. Aber auf Grund meiner zahlreichen Untersuchungen über dieses Insekt dürfte die Möglichkeit gegeben sein, dass wenigstens die Abbauprodukte, die aus solchen Nahrungsstoffen im ersten Stadium der Verdauung entstehen, durch die Wand des Digestionskanals der Seidenraupe in das Blut derselben kommen und dort durch die Blutfermente weiter gespalten werden.

Von grossem Interesse ist die Veränderung der Blutamylase im Laufe der drei Entwicklungsperioden von Bombyx mori. Eine tägliche, verhältnismässig erhebliche Zunahme an Amylaseaktivität erfolgt während des fünften Lebensalters der Larve, und diese Zunahme erreicht in der Zeit des Spinnens des Kokons und der Umwandlung der Larve zur Puppe ein Maximum. Dagegen beobachtet man eine ausgesprochene, allmähliche Abnahme an Tätigkeit der Blutamylase während der Puppenperiode. Was die bei den Schmetterlingen bestimmten amylolytischen Wirkungen des Blutes betrifft, so ist diese viel grösser als die am Ende des Puppenstadiums bestimmten.

Ferner fand ich bestätigt, dass der Hungerzustand bei der Larve eine bedeutende Vermehrung der Amylase hervorbringt.

Es scheint also sehr wahrscheinlich, dass mit den Intensitätsschwankungen des Energiewechsels, der von der Umsetzung des Körperglykogens seinen Ursprung nimmt, auch diese gesetzmässigen Schwankungen des Amylasegehaltes bei Metamorphose bzw. bei Hunger parallel gehen.

Aus den oben erwähnten Data gelange ich zu der Schlussfolgerung, dass die Blutamylase bei Bombyx mori an der Zersetzung des Glykogens und auch zum Teil an der Verdauung der in den Maulbeerblättern enthaltenen Stärke beteiligt ist.

Sehr kompliziert ist das Verhalten der Proteasen. Trotzdem die Wasserstoffionenkonzentration des Blutes von Bombyx mori nahe bei pH 6,8 liegt, zeigt die darin vorkommende Protease zwei pH-Optima, nämlich pH 2,3 und 8,8. Dies ist eine merkwürdige Tatsache. Vielleicht eine der physiologischen Funktionen der Protease-a liegt, wie mir vorkommt, darin, dass dieses Ferment eine Unvollkommenheit der Eiweissverdauung durch den Digestionssaft ergänzt; denn die Protease dieses Saftes wirkt nur bei starker alkalischer Reaktion.

Da sich die Wirkungen der Blutproteasen mit der Metamorphose von Bombyx mori und beim Hunger der Larve regelmässig verändern, scheinen diese Fermente am Eiweissstoffwechsel in ihrem Körper Anteil zu nehmen.

Wenn wir die erhaltenen zahlreichen Daten in Beziehung bringen zu den morphologischen Verwandlungen, die in den verschiedenen Entwicklungsperioden eintreten, dann können wir über das, was wir beobachtet haben, eine interessante Diskussion eröffnen.

Bei Bombyx mori besteht ein grosser Unterschied zwischen der Körperkonstruktion der Larve und der des Schmetterlings. Heftige Umformungen der verschiedenen Gewebe oder Organe müssen infolgedessen während der Zeit der Puppe stattfinden. Nach meinen Erfahrungen scheinen Fermente im Blute mit diesen Umformungen zu tun zu haben. Vor allen Dingen kommen hierbei die Änderungen der Proteasen im Laufe der Entwicklung in Betracht. Denn die Zersetzung bzw. Synthese des Eiweiss ist mit der Auflösung bzw. Neubildung der Organzellen verknüpft. Meine Versuchsresultate weisen darauf hin, dass die Wirkung der Protease-a, deren optimale Wasserstoffionenkonzentration bei pH 2,3 liegt, vom ersten Stadium des fünften Lebensalters bis zur Zeit des Kokonspinnens nach und nach zunimmt und dann sinkt, und ferner, dass im ganzen Zeitraum der Entwicklung diese Fermentwirkung einige Tage vor dem Ausschlüpfen der Imagines am aktivesten ist und dann wieder allmählich sich verringert. Etwas anders verhält sich die Protease-b. Dieses Ferment, welches bei pH 8,8 am besten wirkt, wird während des fünften Lebensalters der Larve von Tag zu Tag schwächer, erreicht sein Minimum in den Tagen des Kokonspinnens, und verstärkt sich dann kurz vor dem Schlüpfen bis zu einem Maximum.

Charakteristisch ist die Erscheinung, dass eine kurz vor dem Schlüpfen stehende Puppe solche maximale Wirkungen beider Proteasen aufweist. Diese Erscheinung, welche wir niemals mit anderen Blutfermenten von Bombyx mori erfahren können, gibt einen wesentlichen Stützpunkt für die Annahme einer Abhängigkeit der Blutproteasen von der Ausbildung der Geschlechtsprodukte bei den Insekten ab.

Es ist aber schwer, den Zusammenhang zwischen den Blutfermenten und den Organumformungen bei Bombyx mori genau zu bestimmen, weil keine eingehenden Arbeiten über den Verlauf der histologischen Veränderungen während der Entwicklung dieses Insekts vorhanden sind.

Die in dieser Untersuchungsreihe über die Enzyme von Bombyx mori mir oft begegneten Befunde, dass einige Fermente des Blutes, wie zum Beispiel Saccharase, Maltase, Lipase und Protease, bei schlecht gewachsenen Larven etwas stärker sind als bei gut gewachsenen, sind meiner Ansicht nach durch zwei Arten von Tatsachen zu erklären, nämlich erstens durch die Ergebnisse meiner zahlreichen Versuche, dass die Blutfermente bei diesem Insekte an den Stoffwechselprozessen in seinem Körper beteiligt sind, und zweitens durch das im allgemeinen bekannte Oberflächengesetz, nach welchem der Energiebedarf bei Tieren sich nach ihren Oberflächenentwicklung richtet.

Immerhin zeigen alle diese Untersuchungen, insbesondere die Versuche, die die Wirkungen der Blutfermente bei Bombyx mori in den verschiedenen Phasen ihrer Entwicklung betreffen, eine Mengen Anhaltspunkte, die für wichtige Funktionen der Oxydationsfermente bei den Stoffwechselvorgängen im Organismus und auch für gesetzmässige Beziehungen dieser Oxydationsfermente und der hydrolytischen Fermente im Körper sprechen. Die Versuche sollen in dieser Richtung sowie auch noch in anderer Beziehung fortgesetzt werden.

Zusammenfassung.

(1) Die Wirkungen der Proteasen im Blute sind bei den Männchen etwas stärker als bei den Weibchen und auch bei schlecht gewachsenen Larven ziemlich höher als bei gut gewachsenen. Hungerzustand bei Larven ruft Abnahme der Proteasenaktivität hervor. Im allgemeinen ist der Proteasengehalt des Blutes der chinesischen und der europäischen Rassen etwas höher als derjenige der japanischen.

(2) Es wurden die Veränderungen der Blutfermente im Laufe der drei Entwicklungsperioden verfolgt. Die Protease-a, die bei pH 2,3 die optimale Aktivität zeigt, wird während des fünften Lebensalters der Larve mit dem Wachstum vermehrt und in den Tagen des Kokonspinnens sehr aktive. Dann sinkt sie und nimmt wieder zu, um ihr Maximum kurz vor dem Ausschlüpfen der Imagines zu erreichen. Hierauf verringert sie sich am Ende der Puppenperiode und im Schmetterlingsstadium allmählich.

Aber die Protease-b, deren optimale Wirkung bei pH 8,8 liegt, wird im fünften Lebensalter nach und nach schwächer und erreicht ihr Minimum etwa in der Zeit des Spinnens des Kokons. Dann vermehrt sie sich gegen Ende der Puppenzeit bis zu einem Maximum und fällt in der Imago wieder ab.

Die eiweissspaltende Kraft der Protease-a ist stets recht viel grösser als diejenige der Protease-b.

(3) Die Wirkung der Blutamylase ist bei den Weibchen immer stärker als bei den Männchen, aber es besteht kein wesentlicher Unterschied zwischen gut gewachsenen und schlecht gewachsenen Larven. Durch Hunger wird die Amylase gesteigert. Die Amylaseaktivität der chinesischen und der europäischen Rassen ist höher als diejenige der japanischen.

Die Amylasewirkung nimmt im fünften Lebensalter der Larve von Tag zu Tag zu und erreicht in der Zeit des Kokonspinnens und des Verpuppens ein Maximum. Während der Periode der Puppenruhe vermindert sich die Schmetterlingsperiode wird sie wieder bedeutend stärker.

(4) Es wurde über die physiologische Bedeutung der Protease und Amylase des Blutes bei Bombyx mori diskutiert und dabei zu der Schlussfolgerung geführt, dass diese Blutfermente in den Stoffwechselvorgängen und auch zum Teil in den Verdauungsprozessen bei diesem Insekte eine wichtige Rolle spielen.

Grossen Dank schulde ich Herrn Prof. Y. Okuda, der mich immer in liebenswürdiger Weise mit Rat und Tat unterstützte.

Über die physiko-chemiche Eigenschaft des Harnstoffnitrats.

Von

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(*Taihoku Kaiserliche Universität, Taiwan, Japan.*)

(Eingegangen am 2. August 1934)

Harnstoffnitrat, das aus je ein Molekül von Harnstoff und Salpetersäure besteht, hat nach mehrere Versuche sich als eine geeignete Düngemittel erwiesen, enthaltend das Stickstoff von Harnstoff- bzw. Salpeterformen. Seine augenscheinliche Überlegenheit dem Harnstoff ist seine kleinere Hygroskopicität. Wegen des leichten Darstellung und Behandlung gibt es solche Hoffnung für diese Verbindung in der Zukunft, dass sie auch als Düngemittel Verwendung finden mag.

Es wurde in vorliegender Arbeit über die verschiedene wichtige Eigenschaften des Harnstoffnitrats studiert.

(1) Wegen kleiner Löslichkeit des Harnstoffnitrats in Salpetersäure ist Harnstoffnitrat aus der gesättigten Wasserlösung Harnstoffes und entsprechenden Menge von konz. Salpetersäure ganz einfach als die Niederschlage zu erhalten.

(2) Der Schmelzpunkt ist 157°C, und das spezifische Gewicht beträgt 1,63.

(3) Die Löslichkeit des Harnstoffnitrats in verschiedenen Lösungsmitteln ist unten angegeben:

a) Löslichkeit des Harnstoffnitrats in Wasser, Ethanol und Methanol.

(Harnsffnitrat Gr. in 100 Gr. Lösung)

	Wasser	Methanol	Ethanol
30°C	26,28	11,65	5,05
35°C	30,01	13,58	6,18
40°C	34,94	15,78	7,46

b) Löslichkeit des Harnstoffnitrats in Salpetersäure.

Konz. von HNO_3 in *N*.	0,1	0,2	0,5	1,0
Löslichkeit	24,7	22,0	18,5	15,6

c) Löslichkeit des Harnstoffnitrats in anderen Lösungsmitteln.

Diese Verbindung ist gar nicht löslich in Äther, Benzol und Chloroform bei gewöhnlicher Temperatur wie bei dem Harnstoff. Dagegen in Azeton bei 30°C ist sie 3,7% löslich.

(4) Die Zähigkeit der Harnstoffnitratlösung im Wasser.

Die Zähigkeit wurde mit Ostwaldviskosimeter gemessen, und als die spezifische Zähigkeit gegen Wasser (=1) angegeben.

a) Zähigkeit der Harnstoffnitratlösung bei verschiedenen Konzentrationen (30°C).

Konz. d. Lösung in Mol.	1,827	1,096	0,914	0,609
Zähigkeit	1,192	1,102	1,065	1,039

b) Zähigkeit der gesättigten Harnstoffnitratlösung bei verschiedenen Temperaturen.

Temp. °C	25	30	35	40	45
Zähigkeit	1,157	1,192	1,236	1,286	1,338

(5) Der Wasserdampfdruck der Harnstoffnitratlösung im Wasser.

Der Wasserdampfdruck der gesättigten Harnstoffnitratlösung im Wasser wurde mittels Isoteniskops ermittelt, und mit dem der Harnstofflösung verglichen. Dadurch hat man die kleinere Hygroskopicität Harnstoffnitrats gegenüber Harnstoff bestätigt.

Temp. °C	Wasserdampfdruck in mm Hg.	
	Harnstoffnitrat	Harnstoff
30	29,1	23,1
35	38,2	29,0
40	49,8	37.7

Studies on Dietary Requirements for Lactation.

II.—Presence of Lactation Promoting Substance in Liver Extract.

By

Waro NAKAHARA and Fumito INUKAI.

[Reprinted from Sci. Pap. I. P. C. R., Vol. 22, pp. 33—38, 1934]

Introduction.

We previously described a synthetic diet which is adequate for excellent growth of young rats, maintenance of good general health, pregnancy and parturition, but is inadequate for successful lactation.(1) This diet has been worked out in order to provide a basal diet for animal experiments aiming at the elucidation of the dietary requirement for lactation, for it is obvious that such a diet as this is prerequisite for a proper approach to the subject. The lactation promoting action of any substance may now be tested by simply adding it to this basal diet.

In the present paper we wish to report our subsequent experiment, which, in our opinion, demonstrates the presence of a lactation promoting substance in liver extract.

Basal Diet and Liver Extract.

The composition of the basal diet in question is as follows:—

Polished rice powder	75 g
Fish protein	10 g
Butter	10 g
McCollum's salt mixture	5 g
Dried yeast	5 g

Liver extract was prepared as follows: 1 kg of mashed fresh beef liver was throughly mixed with water, to which 50 c.c. of 25% sulphuric acid and sufficient amount of 90% ethyl alcohol were added to make the total volume of this mixture 5 litres and the final concentration of alcohol 60%. The extract was pressed out of the mixture, decolorized by means of a small amount of animal charcoal, and neutralized with calcium oxide. It was then concentrated into a suitable volume in the atmosphere of CO_2 gas under reduced pressure and at a temperature below 60°C.

The concentrated extract thus prepared was added to the basal diet at the rate of 3 g per 100 g of basal diet. This amount of extract corresponds to the yield from 100 g of fresh liver.

Breeding Experiment.

The general procedure of the present experiment was the same as that adopted in our previous work. A series of pairs (males and females) of young albino rats, each pair in a separate cage, were maintained on the basal diet, keeping careful observations on the body weight changes, especially of the females. Fifty-eight days after the beginning of the feeding, liver extract was added to the diet for the test group while the control group was continued on the basal diet alone.

The parturition was anticipated by means of observations on growth curves and the general appearance of the females, without having recource to the examination of vaginal smears. As soon as the approaching parturition was detected, the cohabitant male was separated and appropriate quantities of straw and cotton were put in the cage. Feeding and observation were continued with minimum disturbance to the gravid female. The same care was exercised after parturition, and all unnecessary disturbance to the mother rat and her young was avoided.

The success or failure of lactation was determinable within two or three days after parturition. The females suckling the young were continued under observation for three weeks more, i. e., up to the time of weaning the young, and at that time the body weight of the young was noted.

In the case of the failure to suckle the young the mother rat was killed as soon after the death of the young as possible (always within 3 days after parturition), and autopsy was performed in order to ascertain the functional state of the mammary grands.

The results of the experiment are shown in Charts 1 and 2.

Chart 1 represents the growth curves of 6 females composing the test group, which was given supplementary feeding of liver extract. The beginning of this supplementary feeding is marked by the sign +.

P indicates parturition, and the number of black dots following it represent the number of young born. The young which were successfully suckled are indicated by short growth curves starting from the black dots, the terminal point of the growth curve being the body weight at the age of 21 days.

As is evident from the chart, 3 out of 6 females suckled their young and were able to raise them.

The remaining 3, which failed to suckle their young, were killed and their mammary glands examined. It is of importance to note that all these rats showed evidence of active milk secretion, as we shall describe later in this paper.

Chart 2 shows growth curves of 4 control females which were maintained on the basal diet alone throughout. None of these females suckled their young, as may be expected from the result of our previous experiments. Moreover, anatomical examination established that the failure of these control females to suckle their young was directly ascribable to the lack of milk secretion (See a subsequent section of this paper).

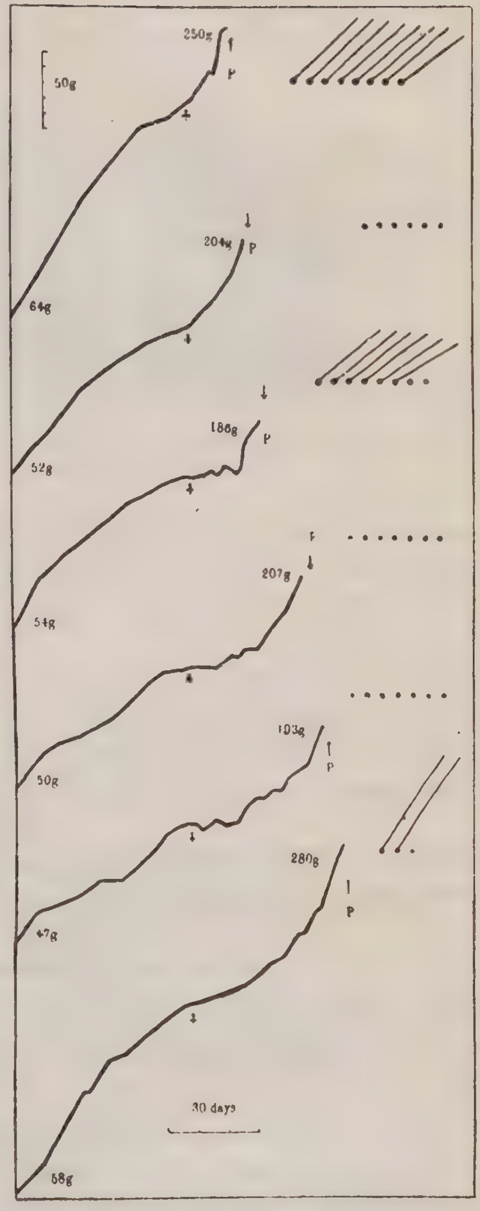

Chart 1—Growth curves of 6 females of the test series supplied with liver extract, showing the details of breeding experiment. Three of the females suckled their young.

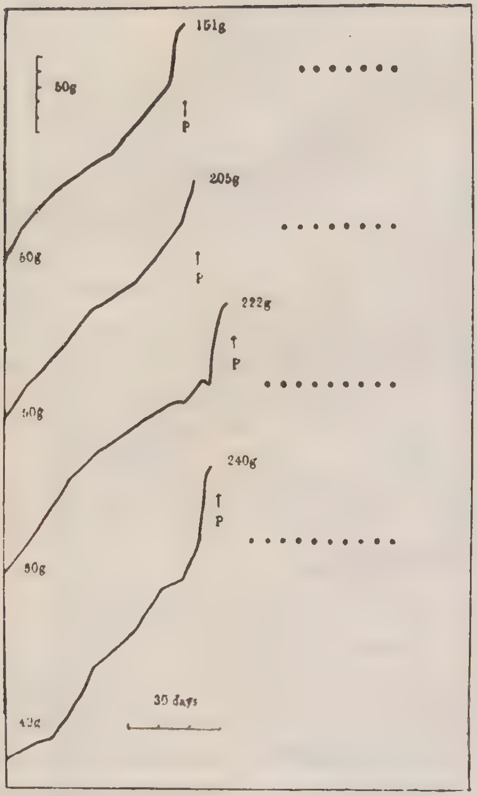

Chart 2—Growth curves of 4 control females, not supplied with liver extract. Young were not suckled by any of these females.

The Growth of the Young.

The growth of the young suckled by the mother rats fed with liver extract was remarkably good.

In Chart 3 we represented all the young belongigning to three litters by a series of vertical columns, the body weight on the 21st day after birth being indicated by the height of the column. The last column, which is shaded to distinguish it from tne others, represents the standard average body weight of rats at the age of 22 days, given in Donaldson's tables.(2)

An inspection of this chart is sufficient to show that the growth of suckling rats in our own series is excellent, so much so that the poorest in growth among them at the age of 21 days is equal in weight to Donaldson's average standard for 22 days old rats. The great majority of our young rats exceeded by far this average standard.

The opening of the eyes of the young took place on about the fourteenth day after birth, which is quite normal.

Comparison of Mammary Glands.

There remains to be described the functional state of the mammary glands of the females which failed to suckle their young in the above experiment.

We have already referred to the fact that in the females supplied with liver axtract, the mammary glands showed evidence of active milk secretion. A gross anatomical examination was sufficient to reveal this evidence. The glandular tissue was well developed and the numerous ducts and tubules of the glands were very clearly marked by their milk contents so that the entire gland gave the appearance of being thickly invested with milky network.

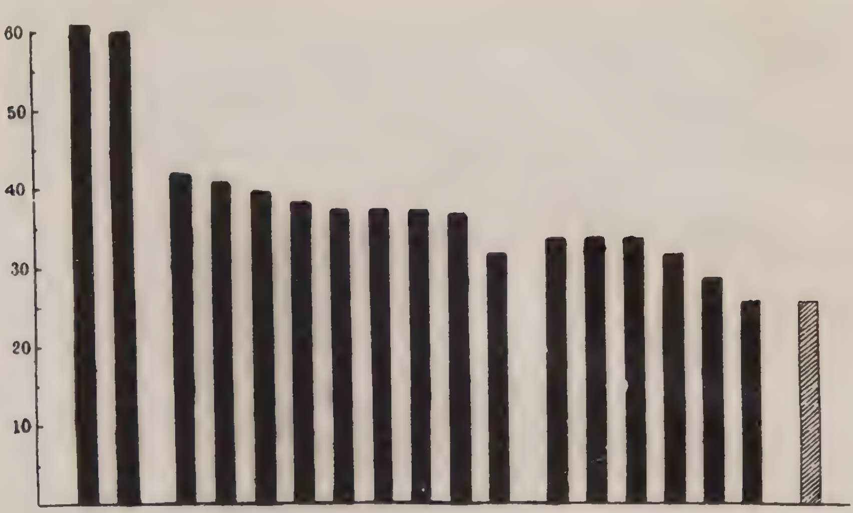

Chart 3—Body weight at 21 days of age of our liver extract series (solid), compared with Donaldson's average standard for 22 days old rats (shaded).

These gross appearances were confirmed by histological examination (Figure 1). Here the interlobular adipose tissue is reduced to the minimum through the expansion of glandular lobules, and the alveoli composing the lobules are all actively secreting.

A striking contrast to the above condition was offered by the general appearance of the mammary glands in rats failing to lactate on our basal diet. The glandular tissue was poorly developed and the ducts and tubules of the glands were either not clearly visible in gross or were only partially detectable by their scanty milk contents.

Histologically, the gland contained much adipose tissue in which were seen relatively small glandular lobules. Most of the alveoli were inactive, alveoli were seen here and there. See Figure 2.

These findings definitely establish that even though these two sets of rats failed to suckle the young, there is no such inhibition of milk secretion in the group supplied with liver extract as constantly occur in controls fed on the basal diet alone.

Comments.

The foregoing data prove that beef liver contains a substance which is capable of strikingly promoting the process of lactation, but does not necessarily demonstrate that this lactation promoting substance is different from vitamin B, especially vitamin B_2. However, the fact that liver is rich in vitamin B_2, which is held by certain authors to be important for lactation, dose not seem to us sufficient to explain our results. Our basal diet which

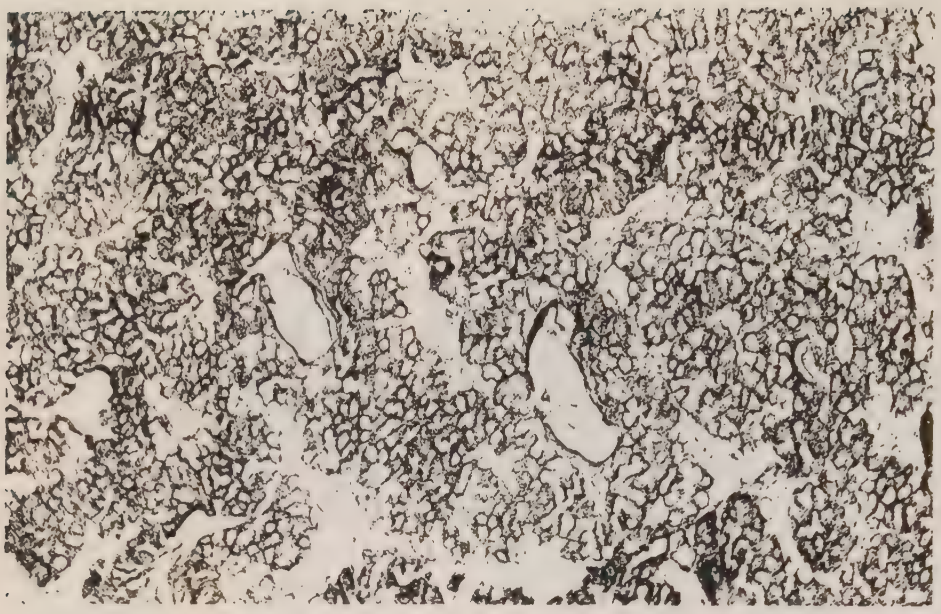

Figure 1—From a rat supplied with liver extract, showing the expansion of glandular lobules composed of actively secreting alveoli.

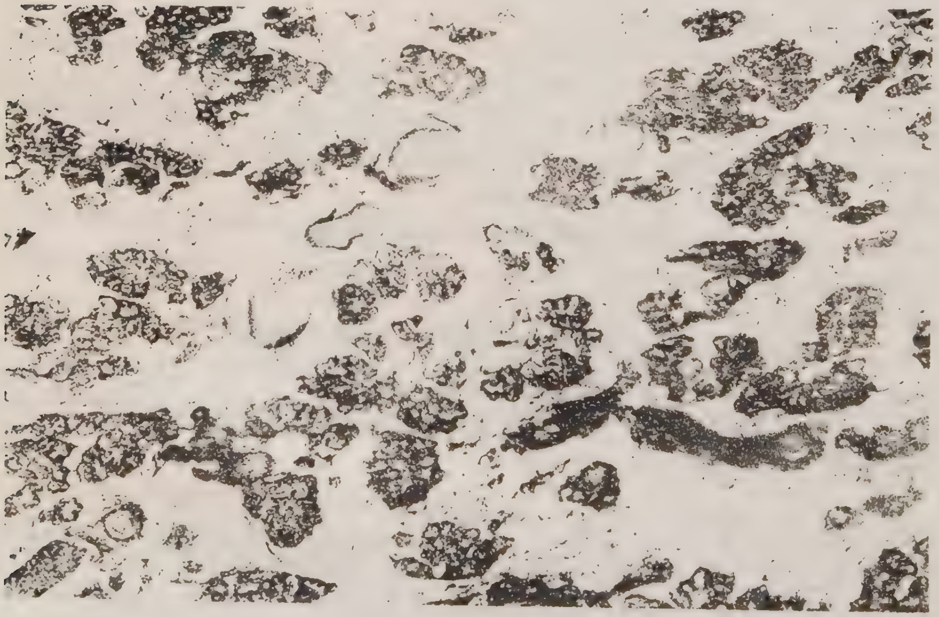

Figure 2—From a rat maintained on basal diet alone. Same magnification as Figure 1.

Photomicrographs of mammary glands (inguinal) of rats failing to suckle the young, killed 3 days after parturition.

is inadequate for successful lactation, contains yeast in the high ratio of 5%, so that the additional vitamin B_2 in liver extract can hardly be expected to bring about any radical improvement in the diet.

It may be suspected that liver contains a new dietary principle allied to but not identical with vitamin B_2, and that this factor plays an important role in the process of lactation. Whether or not this supposed lactation promoting principle is identical with Mapson's(3) growth promoting "physin" is not determinable at this moment.

Conclusion.

Addition of liver extract to a well balanced synthetic diet which is inadequate for lactation, renders this diet largely capable of supporting active lactation, thus demonstrating the existence in liver of a dietary principle which has a distinct lactation promoting action.

We take this opportunity of expressing our cordial thanks to Professor U. Suzuki, M. I. A., for his kindly interest and helpful criticisms.

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(1) W. Nakahara and F. Inukai: Sc. Pap. I. P. C. R., **22**, 301, (1933).
(2) H. H. Donaldson: The Rat, Philadelphia, (1924).
(3) L. W. Mapson: Bioch. J., **26**, 970, (1932).

On the Occurrence of Vitamin B_2 (=Vitamin G) in Sake and Sake-Kasu (Pressed Cake).

By

Fumito INUKAI, Tuneto HIGASI and Waro NAKAHARA.

[Reprinted from Sci. Pap. I. P. C. R., Vol. 24, pp. 113—118, 1934]

The recent increasing interest in vitamin B_2 (or vitamin G) lead us to investigate the possible occurrence of this important vitamin in some characteristically Japanese fermentation products, and as the first step we tested sake (rice wine) and sake-kasu.

A few years ago Takahashi and Rin(1) published their experiments showing the occurrence of both vitamin B_1 and B_2 in sake-kasu. It appears from this paper, however, that they used potato starch in their basal diet. Potato starch is now known to contain traces of growth promoting substance and therefore to be unsuitable as a constituent of a basal diet for vitamin B_2 tests. We ourselves observed a fairly good growth of albino rats on a diet composed of potato starch 75 g, purified fish protein 10 g, butter 10 g, McCollum's salt mixture 5 g and oryzanin 4 c.c.(2)

In the following experiments we used rice starch, tested and proved to be deficient in vitamin B complex, as the source of carbohydrate in basal diet in order to obtain reliable information. We also made use of the adsorption by acid earth as a convenient method of concentrating vitamin B complex, and while acid earth adsorbate may not be commendable for use in human subjects, we found it quite satisfactory for the purpose of animal experiments.

Experiments.

Growth Promoting Action of Sake-Kasu.

Young albino rats of approximately 50 grams body weight were divided into two groups, and one group was maintaind on the following basal diet

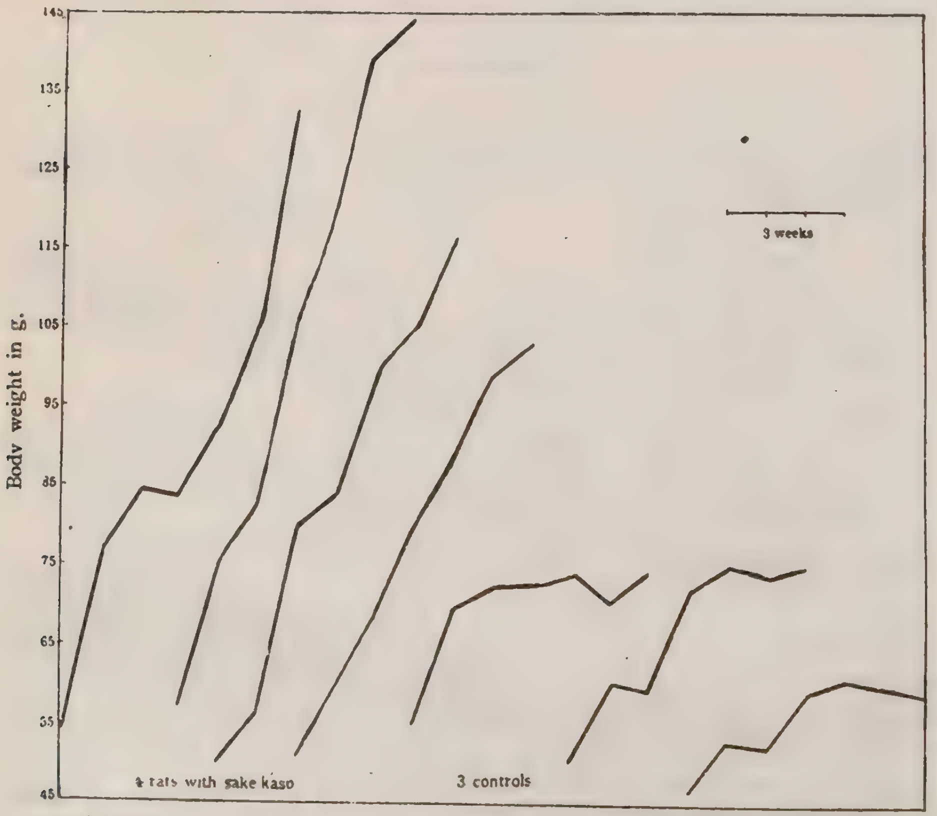

Chart 1.—Growth curves of rats on vitamin B_2 deficient diet with and without sake-kasu supplement.

which is free from vitamin B_2 :—

Polished rice powder	75 g
Fish protein	10 g
Butter	10 g
McCollum's salt mixture	5 g
Oryzanin	4 c.c.

The other group was fed on the same diet as above but with the addition of 8 g (dry weight) of sake-kasu per 100 g of the basal diet.

The result of this experiment, as shown in Chart 1, clearly indicates a marked growth promoting action of sake-kasu.

Adsorption of Vitamin B_2 in Sake-Kasu by Acid Earth.

In view of the well established fact that vitamin B_2 as well as vitamin B_1 are easily and completely adsorbed by a number of adsorbents, we carried out additional tests using acid earth adsorbate of the extracts of sake-kasu.

Sake-kasu was extracted with twice the volume of 60% methyl alcohol containing a small amount of sulphuric acid. To 1 litre of filtered clear extract was added 20 g of white acid earth, and was kept at the temperature of 30°C for 20 minutes with occasional stirring. The acid earth was recovered by filtration and was washed with water, with alcohol, and then with ether. Forty grams of the acid earth so treated was added to 100 g of the same vitamin B_2 deficient diet as used in the preceding experiment.

Since the acid earth adsorbate of sake-kasu extract should carry with it all the available vitamin B_1 along with vitamin B_2, tests were also made for the possible existence of vitamin B_1. The vitamin B_1 deficient basal diet used was the same as the vitamin B_2 deficient diet except that oryzanin was omitted from the composition.

Examples represented in Chart 2 suffice to prove that acid earth adsorbate of sake-kasu is a very potent source of vitamin B_2, but not of antineuritic vitamin B_1.

In Chart 2, Rat No. 1 was maintained on the vitamin B_2 deficient diet containing vitamin B_1 (oryzanin) for three weeks, after which acid earth adsorbate was incorporated into this diet. The typical growth response of the rat following the adsorbate feeding demonstrates the presence in the adsorbate of vitamin B_2.

Rat No. 2 was maintained on the same diet but without the oryzanin supplement, i. e., the diet deficient in both vitamin B_1 and B_2. The addition of the adsorbate to the diet failed to induce growth, and the rat died of typical polyneuritis. This shows that the adsorbate dose not contain antineuritic vitamin B_1.

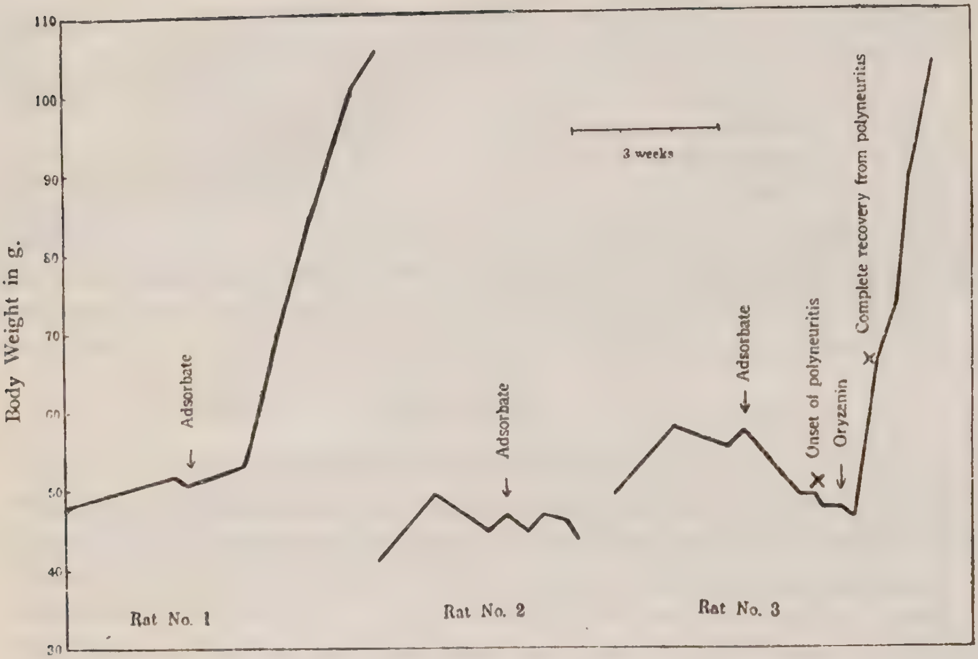

Chart 2.—Vitamin B_1 and B_2 tests of acid earth adsorbate of sake-kasu extract.

Rat No. 3 was fed on the same diet as Rat No. 2, both vitamins B_1 and B_2 being absent. As in the case of Rat No. 2, addition to the diet of the adsorbate called forth no growth response, and the rat manifested typical symptoms of polyneuritis. Oryzanin was, therefore, incorporated into the diet in addition to the adsorbate, upon which the rat recovered rapidly from polyneuritis and at the same time showed very active growth. This demonstrates again that the adsorbate is deficient in vitamin B_1 but is rich in vitamin B_2.

Presence of Vitamin B_2 in Raw Sake.

The presence of vitamin B_2 in sake-kasu naturally leads to the supposition that sake itself may also contain this vitamin.

One litre of sake was acidified by adding 10 c.c. of sulphuric acid and then treated with 20 g of white acid earth, and the adsorbate was separated in the same manner as in the preceding experiment.

Acid earth adsorbate was prepared of a certain popular brand of bottled sake on the market. Tests were performed using the same vitamin B_2 deficient diet as before, and they proved that this adsorbate had no growth promoting action (Chart 3, Rats Nos. 1∼3). Forty grams of adsorbate was added to 100 g of basel diet in these teste.

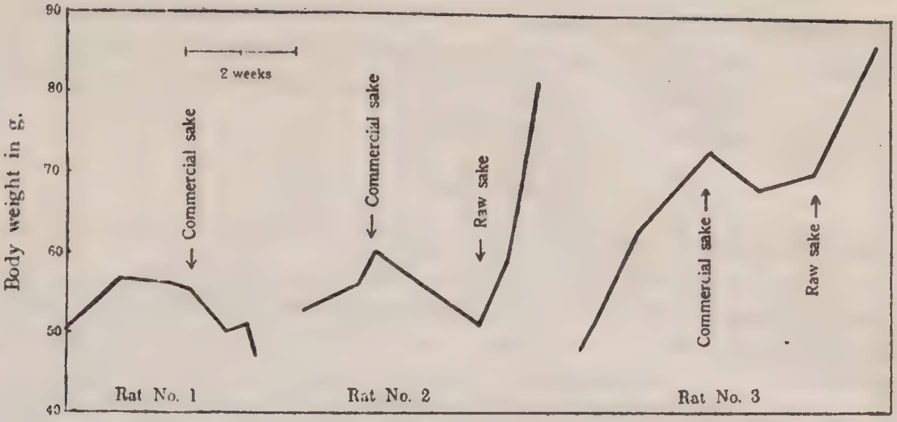

Chart 3.—Vitamin B_2 tests of acid earth adsorbate of raw and commercial samples of sake.

Acid earth adsorbate was prepared also of raw sake ("Ozeki" brewery of Nada) before it was heated to stop further fermentation (the processs called "hiire" among sake brewers) preparatory to placing it on the market. This adsorbate, added in the amount of 40 g per 100 g of basal diet, brought about typical growth respones, indicating the presence of vitamin B_2 (Chart 2, Rats Nos. 2 and 3).

This striking difference between the bottled sake on the market and the raw before the 'hiire" treatment, is probably due to the fact that sake producers treat sake with animal charcoal in order to decolorize the commercial products, with the resultant removal of growth promoting vitamin.

Comments.

The growth promoting vitamin in sake and pressed cake is without doubt derived from sake yeast. It may therefore be of interest to note that 8 g (dry weight) of sake-kasu is sufficient to serve as an adequate source of vitamin B_2. Since only about half the bulk of sake residue is composed of yeast, this amount of sake-kasu may be regarded as representing 4 g of yeast.

We know through our experience that under similar experimental conditions the amount of beer yeast required as an adequate source of vitamin B_2 may be 3 to 5 g. While it is difficult to arrive at a definite conclusion without making accurate quantitative comparison, it would seem safe to asume that sake yeast may not be inferior to beer yeast as to the vitamin B_2 content.

From the point of view of human nutrition it is of interest that sake-kasu, which is often consumed in Japan as an accessory food is now proved to be a rich source of vitamin B_2. As regards the vitamin B_2 content of sake itself, attention should be called to the fact that a bottled sample of a popular brand of sake on the market was found to be devoid of vitamin B_2, probably due to the adsorption method of decolorization to which commercial sake is subjected preparatory to distribution.

Conclusion.

Animal experiments described in this paper demonstrate that sake-kasu and raw sake are good sources of growth promoting vitamin B_2.

Antineuritic vitamin B_1, however, does not seem to be contained in these fermentation products.

The foregoing experiments were conducted under the supervision of Professor U. Suzuki, to whom we desire to express our appreciation. Our thanks are also due to Mr. Nagashima of the Ozeki Brewery, Nada, for his kindness in donating a sample of raw sake.

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(1) E. Takahashi and K. Rin: J. Agr. Chem. Soc. Japan, **5**, 177, (1929).

(2) Unpublished observation.

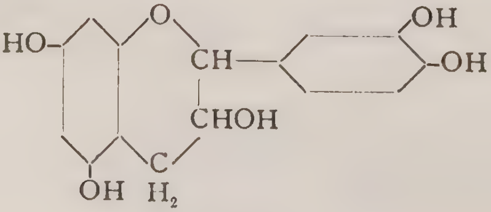
Isolation of a New Catechin, Tea Catechin II or Gallo-Catechin from Green Tea.

By

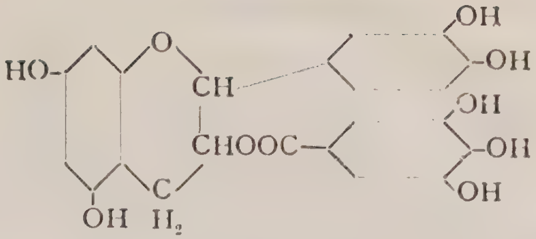
Michiyo Tsujimura

(Received May 15, 1934.)

A few years ago, the author has isolated tea catechin⁽¹⁾ and tea tannin⁽²⁾, ⁽³⁾ from green tea and assigned the following formulae to them.



Tea catechin $C_{15}H_{14}O_6$



Tea tannin $C_{22}H_{18}O_{10}$

Quite recently, a new catechin having the formula $C_{15}H_{14}O_7$ m p 218° has been isolated in crystalline state (Photo. 1) and the name Tea catechin II or Gallo-catechin has been proposed by the author in order to distinguish it from the first catechin, which shall be designated now as Tea catechin I.

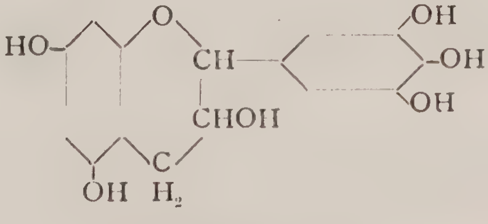
The acetyl derivative of the new catechin forms rhombic crystals (Photo. 2) m p 189～190° and corresponds to the formula $C_{15}H_8O\cdot(OCOCH_3)_6$. The methyl derivative (Photo. 3) obtained from the acetylation product is represented by the formula $C_{15}H_8O(OH)\cdot(OCH_3)_5$ m p 183°.

The acetyl derivative gives phloroglucinol when boiled with concentrated alkali and the methyl derivative forms trimethyl gallic acid by cautiously oxidizing with $KMnO_4$.

The specific rotation of the new catechin is $[\alpha]_D^{15} = -67.5°$ and closely resembles to that of Tea catechin I i. e. $[\alpha]_D^{25} = -69°$.

The absorption spectrum (Photo. 4) is exactly the same with that of the methyl derivative (Photo. 5) but differs somewhat from that of Tea catechin I (Photo. 6).

From these observations, the following formula has been assigned to the new catechin.



Tea catechin II $C_{15}H_{14}O_7$
(Gallo-catechin)

A catechin existing in Malett and Pistacia is presumed by K. Freudenberg(4) to have the same formula. Further, a new tannin $C_{15}H_9O_2(OH)_5$? isolated by Y. Oshima and co-worker(5) from the fresh tea leaves of Formosa seems to be identical with the new catechin of the present author. These points will be investigated later on.

Experimental.

1. *Isolation of New Catechin from Green Tea.*

The material used in this experiment was the green tea of superior quality, prepared in Shizuoka prefecture. It was extracted three times with water at ordinary temperature, and the extract was treated with neutral lead acetate solution until no more precipitate was formed. The greenish precipitate thus produced, was collected, washed with water, and decomposed with 10% sulphuric acid. The reddish yellow filtrate from the lead sulphate was now repeatedly extracted with ethyl acetate, and the extract was evaporated under reduced pressure while passing a current of carbon dioxide. The residue was then dissolved in a small volume of water and after shaking with ether three times, the aqueous layer was separated and evaporated to expel off the ether, and filtered. On keeping the filtrate in an ice box, needle-shaped crystals separated out, which were washed with water, dried on a porous tile and extracted with chloroform in Soxhlet apparatus. The insoluble residue was then dissolved in hot water and filtered while hot. On cooling the filtrate in an ice box, the new catechin separated out: m p 218° (uncorr.). The yield was about 0.25% of the material used.

From inferior green tea no crystals were obtained by the same treatment.

2. *Chief Properties of Tea Catechin II.*

The freshly prepared Tea catechin II is colourless needles, soluble in water, alcohol, ethyl acetate and aceton; difficultly soluble in ether, and insoluble in chloroform and benzen. It is gradually oxidized in the air. The aqueous solution has an astringent taste with sweetish after-taste, and gives violet colour with ferric chloride. It gives also the characteristic phloroglucinol reaction with pine shaving, moistend with concentrated hydrochloric acid, but it gives no precipitate with gelatin. When heated with dilute acid it is converted into a reddish brown substance.

For comparison, the chief reactions of Tea catechin II, Tea catechin I and Tea tannin are given in the following table:

	Tea catechin II	Tea catechin I	Tea tannin
Astringency	weak	weak	strong
Aqueous solution treated with:			
$FeCl_3$	violet	green	blue
Gelatin	no ppt.	no ppt.	white ppt.
heated with:			
dil. acid	reddish brown ppt.	reddish brown ppt.	reddish brown ppt. and gallic acid
Phloroglucinol reaction	positive	positive	positive

3. *Analysis of Tea Catechin II.*

	Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I.	3.547	7.452	1.584	57.30	5.00
II.	3.628	7.638	1.568	57.42	4.84
III.	3.454	7.303	1.496	57.67	4.85
IV.	3.462	7.321	1.384	57.67	4.74
	Calc. for $C_{15}H_{14}O_7$			58.81	4.61

4. *Specific Rotation of Tea Catechin II.*

$$[\alpha]_D = \frac{\alpha \times 100}{c \times l}$$

α :	observed rotation	-0.675
c :	concentration	1% in 95% ethyl alcohol
l :	length of tube in decimeter	1

$$[\alpha]_D^{18} = -\frac{0.675 \times 100}{1 \times 1} = -67.5^\circ$$

5. *Acetyl Derivative.*

Two grams Tea catechin II were dissolved in 10 c.c. acetic acid anhydride, cooled and treated with one drop of concentrated sulphuric acid, the reaction started at once, After several hours the reaction mixture was poured into water and the precipitate thus formed was collected after standing for several hours, washed with water and dried on a porous tile. For purification it was dissolved in hot methyl alcohol and filtered while hot. On cooling the filtrate the acetyl derivative separated as rhombic crystals (Photo. 2), m p 189∼190° (uncorr.).

6. *Analysis of the Acetyl Derivative.*

	Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I.	3.709	7.878	1.513	57.93	4.56
II.	3.218	6.827	1.367	57.86	4.75
	Calc. for $C_{15}H_8O(OCOCH_3)_6$			58.04	4.70

7. *Molecular Weight of the Acetyl Derivative.*

The determination was carried out by Rast's camphor method.[6)]

$$m = \frac{c \times w \times 100}{d\,W}$$

m :	mol wt.	
c :	const.	100
w :	subst. mg	16.1
d :	depress. of m p	5°
W :	solvent mg	225

$$m = \frac{400 \times 16.1 \times 100}{5 \times 225} = 572$$

Calc. for $C_{15}H_8O(OCOCH_3)_6$ 558

8. *Methyl Derivative.*

The methyl derivative of Tea catechin II was prepared from the acetylation product described above.

Two grams of the latter were dissolved in 10 c.c. methyl alcohol, to which 15 c.c. dimethyl sulphate were added and treated with 15 c.c. 50% KOH drop by drop with shaking. The reaction product was poured into water, and after standing for some hours the precipitate was collected, washed with water and dried on a porous tile. For purification it was dissolved in hot methyl alcohol and filtered while hot. On cooling, the methylated product crystallized out (Photo. 3), m p 183° (uncorr.).

9. *Analysis of the Methyl Derivative.*

	Sample (mg).	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I.	3.126	7.285	1.830	63.56	6.55
II.	3.121	7.260	1·775	63.44	6.36
	Calc. for $C_{15}H_8O(OH)(OCH_3)_5$			63.80	6.43

10. *Decomposition of the Acetyl Derivative with Concentrated Potash.*

The acetylated product was boiled with 50% KOH at 180° for 30 minutes. After cooling, the reaction mixture was diluted with water, neutralized with sulphuric acid, filtered and sufficient sodium bicarbonate was added until weakly alkaline, again filtered and extracted with ether. The etherial extract was evaporated and treated with water. From the aqueous solution phloroglucinol separated out in crystalline state; m p 210°.

It gave the characteristic reactions of phloroglucinol; mixed with pure phloroglucinol no depression of melting point was observed.

11. *Oxidation of the Methyl Derivative with Potassium Permanganate.*

The methyl derivative was dissolved in methyl alcohol, heated on a water bath and a strong solution of potassium permanganate was added drop by drop until the red colour remained unchanged on standing, the excess of permanganate was then decomposed with dilute sulphuric acid until slightly acid and extracted with ether. The etherial extract was evaported and the crystalline residue thus obtained was treated with sodium bicarbonate to remove the phenolic substance and recrystallized from hot water. It was identified as trimethyl gallic acid; colourless needles, m p 168°, mixed with pure specimen no depression of melting point was observed.

Analysis :-

	Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
I.	3.251	6.740	1.690	56.54	5.82
II.	3.124	6.495	1.640	56.70	5.87
	Calc. for trimethyl galic acid $(CH_3O)_3C_6H_2CO_2H$			56.58	5.70

12. *Absorption Spectra.*

The absorption spectra of Tea catechin II, its methyl derivative, Tea catechin I, and of Tea tannin are shown in Photos. 4, 5, 6 and 7. (Spectrograph, Hilger's Quartz type ; source of illumination, Hydrogen Geisler tube.)

The author expresses her sincere thanks to Prof. U. Suzuki for his kind direction and encouragement throughout this work, and also thanks to Mr. Funahashi for helping on the elementary analysis.

Literature.

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(3) 〃 : 〃 **7**, 23, (1931).
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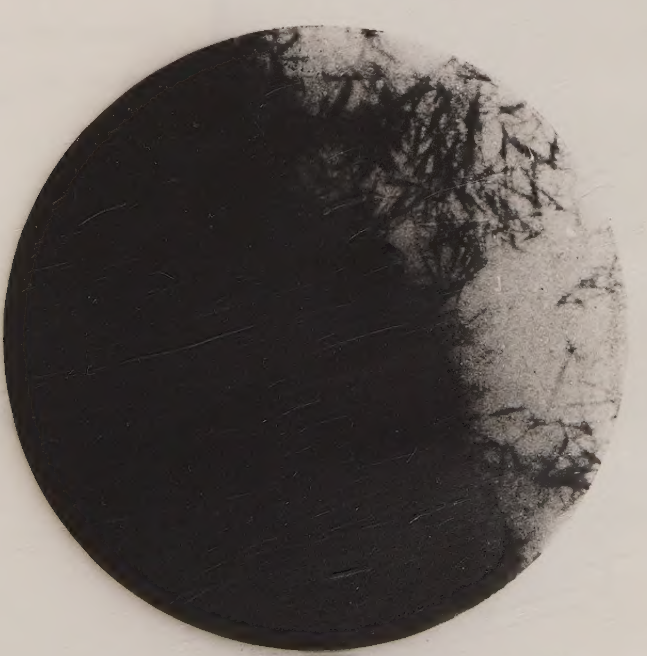

Photo. 1—Tea catechin II from aq. sol.   1 : 100

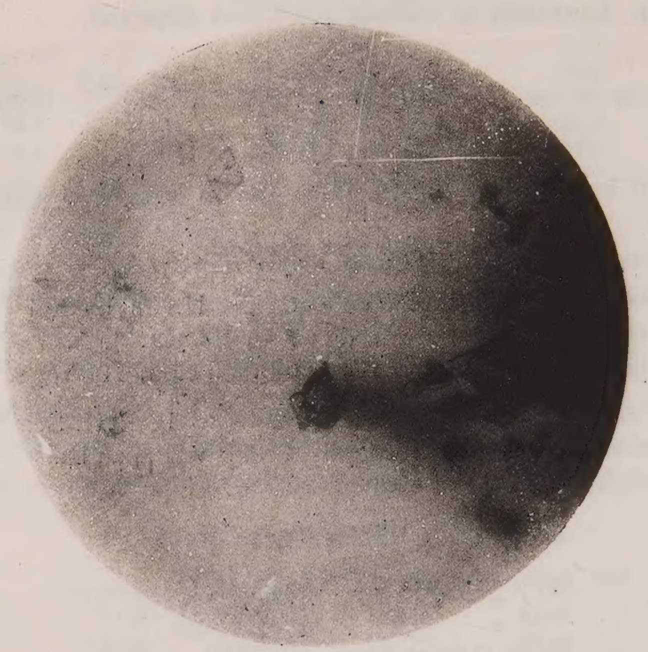

Photo. 2—Acetyl derivative of Tea catechin II from methyl alc. sol. **1 : 100**

Photo. 3—Methyl derivative of Tea catechin II from methyl alc. sol. **1 : 100**

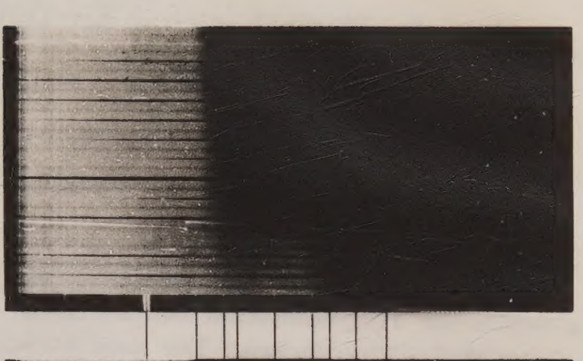

Photo. 4—Tea catechin II, $\frac{1}{1,000}$ mol. in alcohol

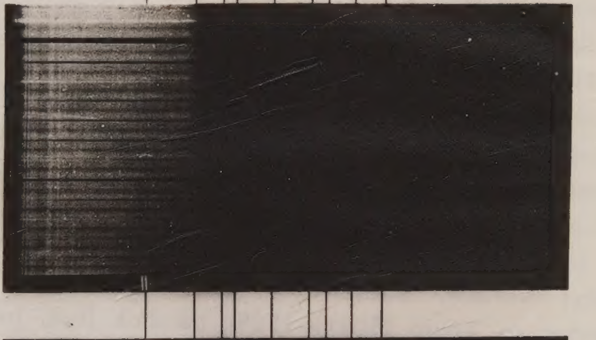

Photo. 5—Methyl derivative of Tea catechin II, $\frac{1}{1,000}$ mol. in alcohol

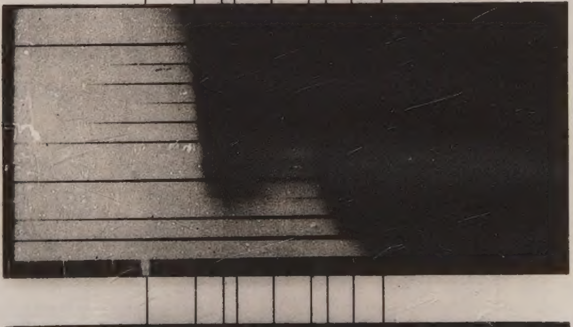

Photo. 6—Tea catechin I, $\frac{1}{1,000}$ mol. in alcohol

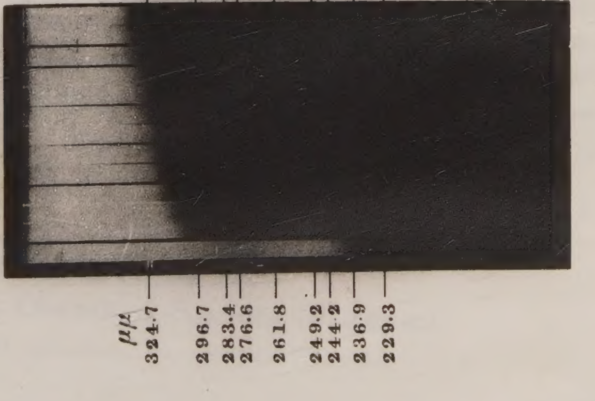

Photo. 7—Tea tannin, $\frac{1}{1,000}$ mol. in alcohol

Über die Hygroskopicität der Düngemitteln.

Von

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(Eingegangen am 10. August 1934)

Es wurde die Hygroskopicität von 20 Arten der Handelsdünger durch die statische Methode verglichen. Die zum Versuche benutzen Düngemitteln sind folgende:

Harnstoff, Harnstoffnitrat, Leunasalpeter I. G., Phosphazote, Ammophos I, Ammophos II, Diammophos I. G., Kalksalpeter I. G., Ammonsulfat I. G., Kalkstickstoff, Ammoniumnitrat, Kalisalpeter, Chilesalpeter, Vit'azote, Sojabohnenkuchen, Superphoshat, Rohphosphat, Rapskuchen, Kalisulfat und Kalichlorid.

Die Temperature und die Feuchtigkeit, denen die Düngemitteln ausgesetzt wurden, wurden aus den Mittelwerten der niederschlagereichsten Monaten in Tokio (1921～1925), nämlich Juni, Juli, August und September, berechnet. Die mittlere Temperatur war 23,5°C, und die mittlere relative Feuchtigkeit war 81,4%. Bei jedem Versuche war die Temperature immer 23,5°C, aber die relative Feuchtigkeit wurde geändert, d. h. 70%, 75%, 81,5 %, 85%, 90% und 95%.

Der gewünschte Wasserdampfdruck wurde mittels des Gemisches von Wasser und Schwefelsäure hergestellt. Die Hygroskopicität wurde durch die Gewichtzunahme der in dem Gläschen gelegten Probe, die in dem Behälter 10 Tage über dem Gemisch von Wasser und Schwefelsäure gehalten wurde, ermittelt. Kalksalpeter, Vit'azote, Phosphazote, Ammoniumnitrat und Leunasalpeter haben sehr starke Hygoskopicität gezeigt. Dagegen haben Kalisalpeter, Harnstoffnitrat, Ammonsulfat und Kalisulfat sehr schwache Hygroscopität gezeigt. Die andere Düngemittel liegen zwischen diesen zwei Gruppen betreffs des Hygroskopicität.